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THE CAPILLARY IN THE HUMAN COCHLEA

I Kirikae, Y Nomura and F Hiraide

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The capillary area of eighteen aged human cochleas was studied using an alkaline phosphatase staining method The technique is described in detail Modes of capillary branching in the spiral lamina were different from turn to turn The vas spirale a loop capillary vessel beneath the tunnel of Corti, was long in the basal turn and short in the upper turn. The spiral lamina of the upper turn was more vascularized than that of the basal turn A morphometric measurement showed that the ratio of capillary density at the areas of 25 mm and 5 mm was 1.5. The significance of the findings is discussed in connection with the vulnerability of the basal turn of the cochlea in sensori-neural deafness.

It has been suspected that disturbances in the inner ear blood circulation are related to some of the inner ear diseases. Experimental work has demonstrated the labyrinth to be damaged temporarily or permanently by circulatory disturbances (Kimura & Perlman, 1956 and 1958 Perlman *et al* 1959 Griffith, 1961 Tsunoo & Perlman, 1964)

It is difficult however to correlate pathologic changes to circulatory failure in the human temporal bone Hemorrhage in the inner ear has only occasionally been reported in the leukemic temporal bone So far sudden deafness, which has long been thought to be of vascular origin, has demonstrated a viral infection of the labyrinth (Lindsay 1959 Schuknecht *et al* 1962) It is extremely difficult to trace blood vessels of the labyrinth in conventional celloidin specimens. The purpose of this paper is to present the morphology of the capillaries of the human cochlear membranous labyrinth and to discuss its funtional significance using a technique we have recently developed.

MATERIAL AND METHOD

Eighteen temporal bones from 14 patients were submitted for this study The age of the patients ranged from 45 to 90 years. The temporal bone was removed and fixed in a 10 per cent solution of formalin methanol The cochlea was dissected without decalcification and stained according to the following procedure

1 A tissue was incubated in the following solution for up to 3 hours at 4 C

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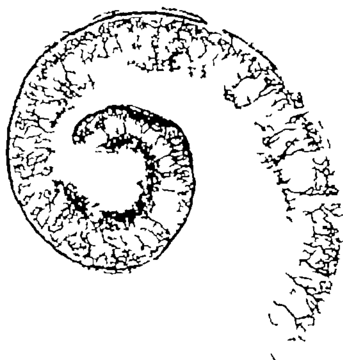


FIG. 2. Capillaries i the pral lamina, limbus, and organ f Corti f the whol left ea Seventy-four-year-old male died f lary geal cancer

More branching of capillaries was seen in the upper turn of the spiral lamina than the basal Morphometry (Weibel, 1963) was performed in an attempt to compare vascular density in different turns. A 25 mm area (500 cps) of the spiral lamina had capillaries of about 1.5 times more than that of the lower basal turn (8000 cps) (Table 2)

TABLE 1 Vas spirale—its characteristics in different turns of the human cochlea

	Basal turn	Upper turn
1. Length of loop	Long	Short
2 Number f anastomosing essels	Few	Many
3. Distance between vas spirale and spiral border near the inner hair cell	Long	Short
4 Anastomosis with essels in the spiral ligament	Not present	Present

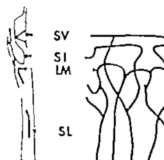


FIG. 1 Schemes of capill ry distribution in the surface preparati n (*right*) and in th ordinarily sectioned specimen (*l ft*) *SV* vas spirale *SI* spiral border vessel near the inner hair cell *LM* capillary in the limbu *SL*, spiral lamina

Naphthol AS-MX phosphate	*1 0 mg*
Dimethylformamide	0 4 ml
Distilled water	*12 0 ml*
0 2 *M* propandiol buffer at pH 8 6	7 6 ml
Fast blue RR	20 0 mg

2 Washed well in running water

3 The tissue was decalcified using a 10 per cent EDTA solution and washed in distilled water

4 Specimens were mounted and flattened in glycerine jelly or PVP medium

RESULT

This paper describes only the spiral lamina vascular system, which is composed of arterioles, venules capillaries running in the spiral lamina and the limbus, and the vas spirale The whole specimen is shown in Fig 2

The vas spirale a looping capillary vessel below the tunnel of Corti, did not run continuously all the way through the cochlea The length of the loops was different from turn to turn In the basal turn it was relatively longer (Fig 3) The longest loop in this series of study measured 2 mm at a 5 mm area (8000 cps) of a cochlea from an 80-year-old patient (No 3074) The loop was short in the upper turn (Fig 4) In other words, there were many anastomosing vessels in the upper turn of the vas spirale and fewer in the basal turn

Near the apical end capillaries were sometimes found further lateral to the vas spirale An anastomosing vessel was present, connecting the spiral lamina vascular system with the spiral ligament vascular system

Another looping capillary was found in the vicinity of the habenula perforata This seemed equivalent to the spiral border vessel near the inner hair cell of Smith s description (1954) The loop ran apart from the vas spirale in the basal turn and close to it in the upper turn (Figs 3 and 4)

The characteristics of the vas spirale in different turns is summarized in Table 1



Fig. 6. Collapse of [illegible] pillaries (arrows) [illegible] the [illegible] piral [illegible] mina. SV [illegible] pirale (out of focu.)

Table 2. *Comparison of capillary density in different turns of the spiral lamina*

	Patient no	Side	Age	Sex	25 mm area/ 5 mm area
1	3074	r	[illegible]	f	2.0
2.	3078	l	81	m	1.9
3.	3069	r	87	f	1.8
4.	3089	l	87	f	1.3
5.	3090	r	79	m	1.2
6.	3090	l	79	m	1.5
7	3091		90	f	1.3
8	3091	l	90	f	1.2
9	3092		87	m	1.0
10	3092	l	87	m	1.4
					1.5 mean

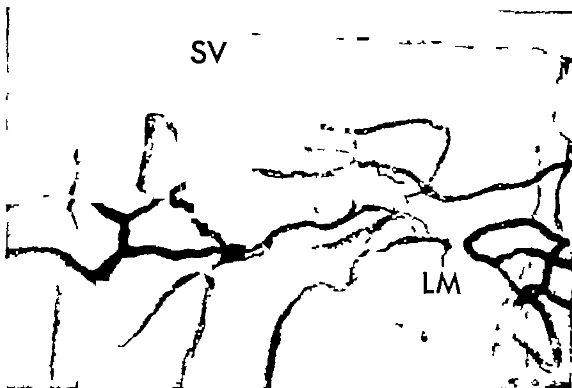


FIG. 3 Capillaries in the basal turn of th ochlea (4 mm are) Eighty seven yea -old f mal A long loop of va pirale (560 μ) *SV* Va spirale *LM* limbus.

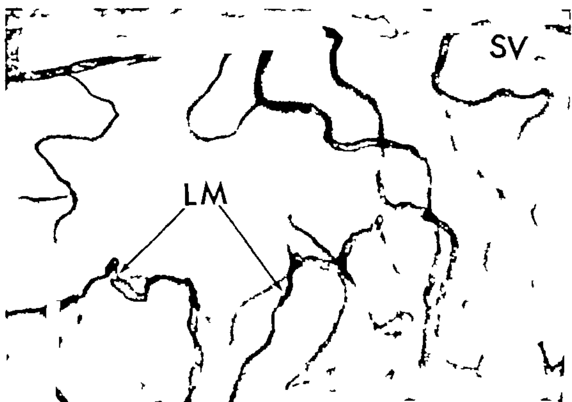


FIG 4 Capill ries f 23 mm rea Th piral l d fici t for l gth f 100 μ SI V piral *LM*, pill ries l th limbus.

discussed, the other runs near the inner hair cell. The present study shows that two spiral vessels run close in the upper turn. This, again, favors the sensory cells in the upper turn, because even if the vas spirale is not functioning, the spiral border vessel near the inner hair cell will compensate a part of the function of the vas spirale such as the diffusion of oxygen.

Morphometry definitely indicated a dense capillary network in the spiral lamina of the upper turn. Further study is being conducted to discover whether or not this is due to aging phenomena.

According to Freygang & Sokoloff (1958) blood flow rates in the cat brain parallel capillarization. Sokoloff (1961) stated that the posterior colliculus, various cortical regions, and geniculate bodies showed maximal capillary density and the highest rates of blood flow. A comparison of measurements of capillary density and succinic dehydrogenase activity in various regions of the cat brain gave a correlation coefficient of 0.88 (Friede *et al.* 1963). Taking account of these facts, scantly blood supply to the organ of Corti, where the metabolic activity is thought to be high, is contradictory if the vas spirale is the only source of nourishment to the organ of Corti. The oxygen tension was high near the stria vascularis. Deeper in the scala media it decreased gradually (Misrahy *et al.* 1958). The organ of Corti is less vascularized and less oxygenated.

To sum up, when circulatory disturbances occur the sensory cells of the basal turn will be damaged more easily than those of the upper turn.

In several conditions, such as ototoxicity, presbycusis, and so on, vulnerability of the basal turn might be due to this anatomical arrangement of the capillaries of the cochlea. *Although mechanisms with which the organ of* Corti is involved are not understood in most circumstances, less blood circulation in the basal turn of the cochlea will definitely delay the functional restoration of damaged sensory cells.

ACKNOWLEDGMENT

We would like to express our thanks to Drs. Amak[illegible] and Kameyama of the Tokyo Yokufukai Geriatric Hospital for their cooperation.

ZUSAMMENFASSUNG

Unter Benutzung von Färbung mit Alkaliphosphatase wurden die Kapillaren an 18 menschlichen Cochlea bei Greisen beobachtet. Die Methode wurde im [illegible]nzelnen beschrieben. Die Art und Weise der kapillaren Abzweigung in der Spiral-Laminae war verschieden. Die Vas spirale, ein schlingenförmiges Kapillargefäss unter dem Cortischen Tunnel, war lang in der Basalwindung und kurz in der Aufwärtswindung. Der Spiral-Lamina der Aufwärtswindung hatte mehr Kapillaren als die der Basalwindung. Morphometrische Messungsmethode ergab, dass die Kapillaren in dem 25-mm-Spiralplattengebiet um das 1,5fache dichter waren [illegible] im 5-mm-Gebiet. Zufolge dieser Befunde wurde weiter die Leicht-Verletzbarkeit der Basalwindung der Cochlea in Sensori-Neural Taubheit besprochen.

The capillary wall was generally smooth Shrinkage and collapse of the capillary wall observed in a cochlea of very short post mortem time were seemingly evidence of a pathology of microcirculation (Fig 5)

DISCUSSION

The present technique demonstrates activity of alkaline phosphatase in the capillary of the cochlear membranous labyrinth It was possible to trace the course of the capillaries, as the surrounding tissue had almost no enzyme activity The significance of staining in the capillary wall is discussed elsewhere (Nomura & Hiraide 1968)

The functions of the capillaries in the spiral lamina vascular system are

1 Supply of nutrient to the organ of Corti Lawrence (1966) has demonstrated the vas spirale as a source of nutrient for the organ of Corti in guinea pigs

2 Maintenance of the function of the limbus The limbus is not only anatomically related to the tectorial membrane, but has been considered to secrete the endolymph (Voldrich 1967)

3 Formation and absorption of the perilymph Bast & Anson (1949) described the presence of a bony dehiscence in the lower shelf of the osseous spiral lamina

Experiments in the human cochlea indicated that the spiral lamina was soaked with the perilymph (Nomura & Schuknecht 1965)

4 Nutrient supply to the nerve fibers in the spiral lamina In addition to nourishment from the cell body nerve fibers are possibly nourished by the surrounding medium and capillaries

Consequently circulatory disturbances in the vascular system will influence most of the cochlear compartments.

It is interesting to know that modes of distribution of the capillaries are different from turn to turn When circulatory failure occurs, a long vas spirale in the basal turn is not beneficial to the organ of Corti As already mentioned, the longest loop was 2 mm long in this series of study This indicates that sensory cells serving about half of an octave are nourished by a single loop of vas spirale provided the vessel has a role as the source of nutrients for the organ of Corti

Alkaline phosphatase reaction in the venous capillary in the membranous cochlea of the guinea pig was either very weak or showed no activity (Nomura & Hiraide 1968) In a loop the spiral vessel of the guinea pig had varying degrees of enzyme activity This indicates that a segmental loop serves both as an arterial and venous capillary Arterial blood enters into a loop supplies oxygen transports metabolites to and from the tissue and finally leaves the loop as venous blood from the other end

As described by Smith, two discontinuous spiral borders are recognized in the organ of Corti and its vicinity One is the vas spirale we have just

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EXPERIMENTAL OTOSCLEROSIS

Its Causation by Ionizing Radiations

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Foci f osteogenesis of otosclerotic appearance were obtained in the nchondral layer of the otic capsule of dogs, using ionizing radiations. In the first dog 5 sessions were carried out over 17 days. The total dose given was 8330 rads nd 7920 rads in th right and left ear respectively The second dog underwent 6 sessions over 22 days and received a total dose of 7920 rad and 5280 rads i the ight and left ear respectively The petromastoid portions f the temporals were fixed in 10 formaldehyd and decalcified in 5% aqueous solution of trichloroacetic acid The following hist logic techniques were performed H & E, Wilder PAS and Scarlach red. I the e chondral layer of th otic capsules f th first d g 3 otosclerotic foci were foun l in the walls of th internal auditory duct a d of the cochlea I the second dog a diffuse osteogeni reacti n was observed It is believed that the osteogeni foci obtained fulfil the requirements set ut by various authors as demonstrati of experimental otosclerotic lesions. It is furthermore contended that such foci are repair areas in a d italized enchondral layer with exten ive zones of necro is.

Few publications are availal le on the experimental causation of otosclerosis. Wittmaack (1930) reported on the results of experiments carried out personally over several years in the course of which he provoked a venous sta is in the otic capsule of the chicken obtaining histologic images which be regarded as coincident with human otosclerosis. Mayer (1930) refuted Wittmaack pointing out the absence of the characteristic arrangement in foci and that the images contended as pathologic were r utine findings in normal chickens. Weber (1930) reported on experiments during which he attempted the causation of otosclerosis by inducing in dogs systemic meta bolic alteration similar to that present in Recklinghausen s fibrocystic osteitis or that seen—according to the author—in Osteogenesis imperfecta However the evidence submitted was not convincing Kralnz (1936) carried out work along similar lines, inducing experimental poisoning with Viganiol in rats with likewise negative results. On the whole, only the periostal layer was affected by the metabolic alterations, with the production at this level of

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Fig. 1. F rst dog, left ear. Enchondral layer showing bone necrosi revealed by the presence of lensh reas of empty lacunae, some f them indicated by rrows. H vers channel with narrowing nd eventual bliteration of thei lumina by concentric appos tio f basophilic material may be seen. H & E. 190

Fig. 2. First dog, left ea . Otosclerosis-l ke osteogenetic focus. Not that the focus ituated in devitalized one of the enchondral layer. H & E. 30

Figs. 3, 4 nd 5. First dog, right ea . D erse eval tio stages of osteogenetic foci showing marked histological similarities ith human otosclerotic foci. H & E. 30

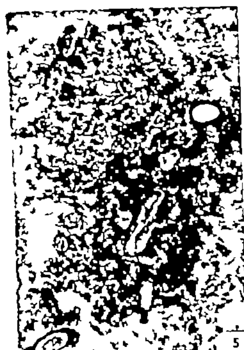
lesions similar to fibrous dysplasia the enchondral layer remaining uninvolved. Lastly Bellucci & Wolff (1960) using electro-coagulation provoked vascular lesions in the otic capsule of animals "with some of the features of human otosclerosis. Upon reviewing material from experiments in rats, intended to cause osteomyelitis in their diaphyses we noticed areas of bone necrosis invaded by foci of osteogenesis similar to those seen in man. This observation prompted us to carry out experiments at the level of the otic capsule itself using various injuring agents. At an early phase chemical agents (sodium morrhuate) and physical ones (ultrasound) were employed in rabbits. Results were variable, attention being drawn to the fact that with the ultrasound there appeared areas of devitalized bone tissue with fatty degeneration of the osteocyte and a moderate osteogenesis at the level of the marrow spaces. Kristensen & Balslev-Jørgensen (1967) found a relationship between otosclerosis and irradiation and suggested that otosclerosis might be brought about experimentally by using radiation as an injuring agent. We have taken up their suggestion the following being a report of the results obtained in dogs.

MATERIAL AND METHODS

The inner ear of two adult dogs was irradiated with a Cobalt 60 beam therapy unit using a single right lateral 4 × 4 cm field. The animals were treated twice weekly under general anesthesia with sodium pentobarbital intraperitoneally. The first dog 3 year old and weighing 4 kg received 1670 rads to the right ear and 1100 rads to the left per treatment. This dog died on the 18th day as a result of cerebral radiation injury (necropsy finding) after having received a total dose of 8330 rads to the right ear and 5500 rads to the left in 2½ weeks. The second animal 4 years old and weighing 6 kg was treated in the same way but received a lower dose per treatment 1320 rads and 880 rads to the right and left ear respectively. The total dose was 7920 rads and 5280 rads in 3½ weeks. The animal was sacrificed 48 hours after the last treatment. The temporal bones of both dogs were decalcified in 5% trichloroacetic acid following fixation in 10% formaldehyde. The petrous bones were sectioned perpendicularly to the large axis and the fragments of otic capsule thus obtained were embedded in paraffin blocks, other fragments being set aside for frozen sections. The following staining techniques were used H E, PAS, Wilder and Scarlach red. Six labyrinthine capsules of normal adult dogs were used as controls.

RESULTS

In the otic capsules of the normal adult dogs it was possible to observe a vitalized enchondral layer patent blood vessels and a few empty lacunae the number of cartilaginous remnants being smaller than in the human enchondral layer. Fatty degeneration of the osteocyte was of rare occur


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6


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8

rence On other hand the irradiated animals exhibited marked pathological alterations. In the otic capsules of the first irradiated dog there were observed areas of bone necrosis involving the enchondral and periostal layers, recognizable by the presence of extensive areas of empty lacunae The techniques for demonstration of fats revealed the existence of sudanophilia in the osteocyte and in the empty lacunae a sign of fatty degeneration at that site Havers canals were seen smaller in diameter resulting from apposition of an intensely basophilic substance leading to eventual vascular obliteration due to extrinsic compression On the whole, these pathologic alterations resulted in a histologic pattern similar to that observed in the normal human otic capsule (Fig 1) As a salient feature stress is laid on the appearance of osteogenetic foci of an "otosclerotic" aspect (Fig 2) The osteogenic areas derived from the invasion of the enchondral layer by blood vessels stemming from the periostal layer The three foci observed were entirely confined to the enchondral layer One of them (left ear) lay within the wall of the internal auditory duct and the remaining two (right ear) within the cochlear wall Following decalcification one of the right ear focus could be seen with the naked eye on the cut surface as a minute whitish area against the greyish background of the enchondral layer Microscopically the newly formed bone tissue was made up of bone trabecules arranged in a disorderly erratic, non laminillar fashion separated by spaces occupied by connective vascular tissue The size of these spaces was variable depending on the existing osteoblastic activity In the oldest focus the bone tissue was of a sclerous appearance its vitality being nevertheless preserved In the more recent foci it was possible to observe a predominance of bone resorption together with an intense vascularization of the area concerned (Figs. 3 4 5 and 6) These osteogenic areas were markedly PAS-positive the more so in the oldest focus With the Wilder stain an erratic, non reshaped arrangement of the collagenous fibrils was seen the pattern assumed being that of bone tissue of inter-crossed fibers. These areas were in contrast with the adjacent bone tissue characterized by a laminillar arrangement of its fibers

In sections deriving from the second dog there was a diffuse osteogenic activity involving all the layers consisting of overlapping of young bone

Fig. 6 Detail f ph t micrograph 5, h wi g th n wly f rmed bo ti su t scl rotic-lik l ading and substituti g the devit lized nch ndral layer The focu l lea ly d limited. H & E. 80

Fig 7 Second dog, l ft a Not th marked osteog ic cti ty rou d th blood essel with fi g r lik images Ext n i rea f mpty l cu may be observed H & E. 80

Fig 8 Second dog, left Osteog netic rea developed in devit lized n hondral l ye N t the multiplicati r f wid lum n ha ral n b n ls in di g th ench ndral l y from th periostal H & E. 80

the effect of heavy irradiation of adult bone tissue is the production of an aseptic bone necrosis. The necrosed zones—recognizable by the presence of empty lacunae—appear arranged in irregular areas rarely is the demarcation between necrosed and live tissue definitely clear The sequestration of necrosed bone does not take place, except for the occurrence of secondary infection. There is no general agreement on the causative mechanism of the necrosis.

Our own belief in agreement with Trueta (1963) is that the desintegration products of the necrotic cells excite a reparative osteogenesis originating in the periostal vessels. Hence we contend that the osteogenic foci may be interpreted as repair areas at the level of a partially necrosed enchondral layer For the development of the osteogenic foci, the occurence of severe necrosis in the otic capsule is an essential pre-requisite. This contention is based on the fact that in the second animal there appeared no clear-cut foci, but rather a diffuse osteogenic reaction a finding, we believe due to the absence of severe necrosis. Very likely failure in obtaining necrosis may be imputed to the administration of a somewhat smaller more fractioned dose [1]

ZUSAMMENFASSUNG

Die Verfasser erzeugten unter Verwendung ionisierender Strahlungen osteoge ische Zo en otosklerotischen Aussehens in der nchondralen Schi ht der Labyrinthkapseln bei Hunden Das rste Versuchstie wurde 5mal in 12 T gen bestrahlt, wobei di gesamt Dosis 8330 r im rechten und 7920 r im linken Ohr betrug. Das zweite Versuchstier wurd 6mal in 22 Tagen bestrahlt In diesem F ll betrug die gesamte Dosis 7920 im rechten und 5280 im linken Ohr Die t mporalen K ochen wurden in 10% Formaldehyd fi iert und mittels einer 5% igen Trichloressigsäure entkalkt. Folge d histologisch Techniken wurden angewandt H E., Wilder PAS und Scharlach rot. I der nchondralen Schicht der Labyrinthkapseln des ersten Hundes wurden 3 *otosklerotische* Zonen a der Wand des Inne ohr kanals und der Schnecke gefunden Beim zw iten H nd wurde I e diffuse osteogenische Reaktion beobachtet. Die Verfasser glauben das die osteogenischen Zonen d Vorderungen entsprech n, di verschi d e Autoren als ma gebend für experimentell Otosklerose betra hten und sind usserdem der M inung, dass di osteoge ischen Herd Erneuerungsg biet darst llen di sich in iner lebensunfähigen enchondralen Schicht mit ausgedehnten Nekroseherden entwickeln

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[1] After sending the originals, third dog was irradiated with total dose f 10,000 i the right ear nd 6000 i the left. W found osteogenic focu of otosclerotic pattern. This focus w altogether simila t those described in the paper and wa situated i the enchondral layer of the right labyrinthine capsule.

tissue or of osteoid tissue in areas formerly reabsorbed by osteolysis. These areas of newly formed bone were separated from one another by old bone tissue While no osteogenetic foci similar to those seen in the first dog were found formation of new bone was evident and the presence of finger like images consisting of a central vessel from which satellite vessels surrounded by "blue mantles radiated was of frequent occurrence (Figs 7 and 8)

It should be pointed out that these images have been regarded by Nager (1939) as representing the initial stage of otosclerosis.

DISCUSSION

To date none of the attempts to produce experimental otosclerosis have attained any reasonable degree of success Available work in the field has failed to meet the requirements which identify the otosclerosis lesions as stated by Altmann (1960) Nager (1939) and Mayer (1932)

(*a*) Lesions should be focal non-diffuse and confined to the labyrinthine capsule

(*b*) The assumed causative factor should be capable of producing foci not only in the area of the oval window but in all the typical localization, including the internal auditory duct and the semicircular ducts

(*c*) Due regard should be given to the difference, so far as the histologic picture is concerned between the human and the animal otic capsule only that of monkeys and to some extent, of dogs bear resemblance with the human otic capsule

We believe that our results colsely meet such requirements Firstly we chose the dog as the experimental animal under normal conditions the *otic capsule of the dog is rather similar to that of man and after heavy* irradiation it assumes a devitalized appearance (areas of empty lacunae fatty degeneration of the osteocyte) *In accordance with Nager (1939)* and our own studies (1961 and 1966) this devitalized aspect is precisely one of the outstanding features of the enchondral layer of the human otic capsule Secondly we obtained focal lesions, neatly distinct from the adjoining bone tissue. Thirdly the foci, which apparently derived originally from the vessels stemming from the periostal layer developed exclusively in the enchondral layer one of them in the wall of the internal auditory duct and the others in the cochlea It is worth stressing that no cases of spontaneous otosclerosis in animals have so far been reported according to Weber (1930) and Kristensen & Balslev-Jørgensen (1967) Furthermore in accordance with our pathogenic conception of otosclerosis, mammals cannot have it as their otic capsule is always well vitalized As a result, one should discard the possibility of spontaneous otosclerosis in an irradiated dog or that irradiation has activated latent otosclerotic foci (Kristensen & Balslev Jørgensen 1967) It is common knowledge (Dunlap 1966 Moss, 1959) that

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RESPIRATION OF THE COCHLEA AND FUNCTION

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Brief reversible depressi n of cochlear respiration was produced in the gui ea pig with 0.013 cc of 0.2 mM sodium cyanide perfused int the scala tympani of th basal turn for 80 seconds. Brief reversible depression f cochlear respirati n was also produced by systemic hypoxia (respiration with 5% xygen) When cyanide perf si n was combined with hypoxia, cochlear respiration and function were more severely ffected The ffects f cy nid a d hypoxia on the terminal cytochrome o id se f the respiratory chain of enzymes in mitochondria are reviewed.

Investigations of the function of cochlear cells are guided by knowledge of the reaction of oxygen and metabolic inhibitors with respiratory enzymes. The rate of cell respiration for normal function reflected in oxygen utilization and reduction of pyridine nucleotide varies widely For cochlear cells it can be defined as the lowest level of oxygen concentration of the blood and the slowest blood flow rate that is compatible with normal function.

The activity of the respiratory enzymes (i.e. the terminal cytochrome oxidase) can be controlled by graded hypoxia or by enzyme poisons. In the experiments described below respiration and function of the cochlear cells were depressed in this manner To a id systemic effects, the enzyme poisons (cyanide and azide) were perfused into the cochlea.

METHOD

In addition to previously described procedures, perfusion of the perilymphatic space in the guinea pig was accomplished by a 200 μ diameter polyethylene cannula tightly fixed in the scala tympani of the basal turn. Another hole near the helicotrema allowed the perfusion fluid to escape Perfusion was maintained for 80 seconds at controlled rates with a Harvard perfusion pump Perfu ion fluids included 0 02, 0.2, and 2.0 mM sodium cyanide in Ringer' soluti n, and 0 15 and 1.5 mM sodium azide in Ringer s solution. Systemic an xia was produced by respiration with 5% oxygen. The effect f pH changes on cochlear function was examined with Ringer's solution

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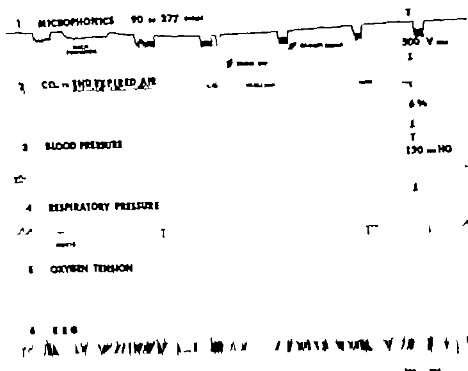


Fig. 1 The reversible effect of i fusion of 0.013 cc f sodi m cyanide (0.2 mM) i t the scal tympani of the basal t rn delivered i 60 seconds on cochlea microphonic output for st ined 277 c i tone t 90 dB. N ystematic ffect are produced nd stri l blood flow is not cha ged. Not 1 A drop i microphonic output duri g i fusion is d to slight temporary increase in perilymph pressure Not 2. Airborne sound from the camera nd light source i the open bulla ppresses (briefly nd revers bly) the conduc tion system stimul ted by the sound deli ered through t bi g int the xternal canal

the cyanide and azide solutions used exceeded this alkalinity Thus, the changes in microphonic output produced with these poisons could be assessed

The next problem was to explore the concentration of cyanide and azide needed to alter the microphonic response in a reversible manner The duration f injections was also important in this respect With 60 seconds of perfusion at 0 01 cc/min, a quantity w s delivered so that the normal perilymph of the scala tympani was replaced by the perfusion fluid. For brief reversible effects on the microphonic output, a concentration of 0.2 mM sodium cyanide was found effective. Solutions of Na cyanide at 0 02 mM and sodium azide at 0 15 and 1.5 mM did not effect microphonic output The reversible effect of cyanide on microphonic outputs is indicated in Table 1 and in Fig 1 An initial RMS value of 390 μ volts drops to 265 μV one minute after infusion at pt. Recovery is complete in seven

TABLE 1 *Effect of NaCN and 5% oxygen on microphonics (RMS) blood flow and oxygen tension*

Changes in blood flow and oxygen tension expressed in per cent of base line

		Minutes after				
Time	Base line	1	2.5	4	5.5	7
0 mM NaCN perfusion of 0 014 cc						
Microphonics	390	265	245	323	355	390
Blood flow	100	120	110	108	98	104
Oxygen tension	100	100	100	100	100	100

			Minutes after		
Time	Base line	During	1	3	5
5% O inhalation					
Microphonics	340	290	340	340	340
Blood flow	100	137	144	121	108
Oxygen tension	100	28	38	0	90
Both procedures					
Microphonics	325	160	160	300	325
Blood flow	100	121	144	124	110
Oxygen tension	100	63	72	90	95

of various pH up to 8 8 perfused into the perilymph This corresponded to the alkalinity of the strongest (2 0 mM) sodium cyanide solution used for cochlear perfusion.

RESULT

A number of problems are involved in the delivery of these respiratory enzyme poisons directly into the perilymph The site of injection is impor tant Injection into the *scala vestibuli* may damage Reissner s membrane with sudden permanent loss of microphonic response The rate of injection is important since too rapid flow results in increased pressure in the coch lea This affects basilar membrane as well as footplate vibration and thus temporarily reduces microphonic output With fluid moving at a rate of 0 01 cc/min into the scala tympani of the basal turn and out through the apical turn a temporary slight depression in microphonic was produced with no change in *strial blood flow Injections at this rate* could be repeated many times without damage The fluid used for solution of cyanide and azide is important No changes in microphonic response were *noted* when mammalian Ringer s solution was perfused in this manner The alkaline nature of cyanide and azide solutions required that the effect of pH changes of the Ringer s solution be examined No alteration in microphonic response was seen when Ringer s solution up to a pH of 8 8 was perfused None of

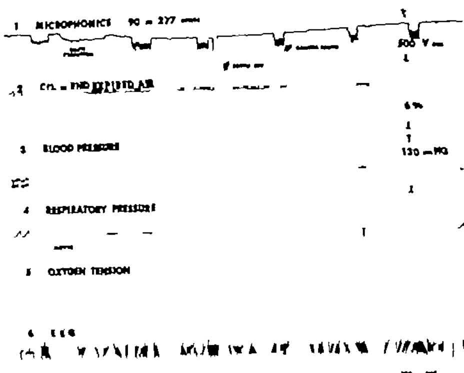


FIG. 1 The reversible effect of infusion of 0.013 cc of sodium cyanide (0.2 mM) into the scala tympani of the basal turn delivered in 80 seconds on cochlear microphonic output for a sustained 277 cycle tone at 90 dB. No systematic effects are produced and strial blood flow is not changed. *Note 1* A drop in microphonic output during infusion is due to slight temporary increase in perilymph pressure. *Note 2.* Airborne sound from the camera and light source to the open bulla depresses (briefly and reversibly) the conduction system stimulated by the sound delivered through tubing into the external canal.

the cyanide and azide solutions used exceeded this alkalinity. Thus, the changes in microphonic output produced with these poisons could be assessed.

The next problem was to explore the concentration of cyanide and azide needed to alter the microphonic response in a reversible manner. The duration of injections was also important in this respect. With 80 seconds of perfusion at 0.01 cc/min, a quantity was delivered so that the normal perilymph of the scala tympani was replaced by the perfusion fluid. For brief reversible effects on the microphonic output, a concentration of 0.2 mM sodium cyanide was found *effective*. Solutions of Na cyanide at 0.02 mM and sodium azide at 0.15 and 1.5 mM did not effect microphonic output. The reversible effect of cyanide on microphonic outputs is indicated in Table 1 and in Fig. 1. An *initial* RMS value of 300 μ volts drops to 265 μV one minute after infusion stops. Recovery is complete in seven

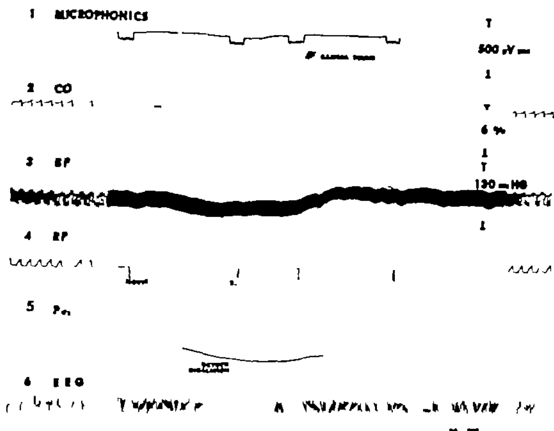


FIG 2 The effect of systemic hypoxia (5% oxygen breathing)

minutes. Simultaneous measurement of blood flow velocity in the stria and of perilymph oxygen tension revealed no significant change. Perfusion with higher concentration of cyanide (2 0 mM) produced a marked more sustained drop in microphonic output Unlike the 0 2 mM solutions, these higher concentrations of cyanide directly effect the performance of the polarographic electrodes by producing small farradic currents so that simultaneous values of perilymph oxygen tension could not be recorded by this method

A significant reduction in oxygen delivery to the cochlea, to produce small reversible changes in perilymph oxygen tension and in microphonic output, was found possible by respiring the animal with 5% oxygen

Other effects of hypoxia are also recorded such as flattening of the EEG, increased cochlear blood flow drop in carotid blood pressure and in CO_2 of end expired air

Thus inhalation of 5% oxygen for 120 seconds results in a reversible drop in microphonic output from an initial RMS value of 340 μV to 290 μV Normal values are regained one minute after return to air breathing (20% oxygen) (Fig 2) Respiration with 5% oxygen for five minutes does not further increase the effect At the same time strial blood flow is increased 37% Flow returns to normal five minutes after resumption of air breathing Perilymph oxygen tension drops to 20% of normal when the animal is respired with 5% oxygen and returns to normal level five minutes after resuming air breathing

1 MICROPHONICS
100
2 CO
6 %
3 EP
4 AP
5 P_{O_2}
6 EEG

Fig. 3. The more severe but reversible effect of combining local cyanide infusion with systemic hypoxia on cochlear function (microphonic output)

The additive effect of cochlear hypoxia and cyanide was noted when inhalation for two minutes of 5% O immediately followed perfusion of the scala tympani with cyanide. Microphonic output dropped from an initial value of 325 μV to 160 μV. Normal values were regained five minutes after air breathing was resumed.

Changes in cochlear blood flow were similar to those produced by hypoxia alone. However, polarographic values for perilymph oxygen tension were less depressed than with hypoxia. Changes in other parameters monitored were similar to those produced by 5% O_2 inhalation (Fig. 3).

DISCUSSION

A. Cyanide has been used widely as a metabolic inhibitor to alter function and oxygen uptake of excised nerves.

Details of the effect of cyanide on the cochlea are limited. Davis (195) report that small doses of cyanide in anyone of the three scala promptly abolished the cochlear microphonics, endolymphatic potential, and action potentials. Rapid (25 minutes) irreversible deterioration of the microphonic

Konishi & Kelsey (1968) found that ten microliters of N CN (50 m. moles) perfused into the scala tympani in 3 to 5 minutes promptly caused severe drop in cochlear microphonics, action potentials, endolymphatic potentials and probably depolarization of the cells in the organ of Corti.

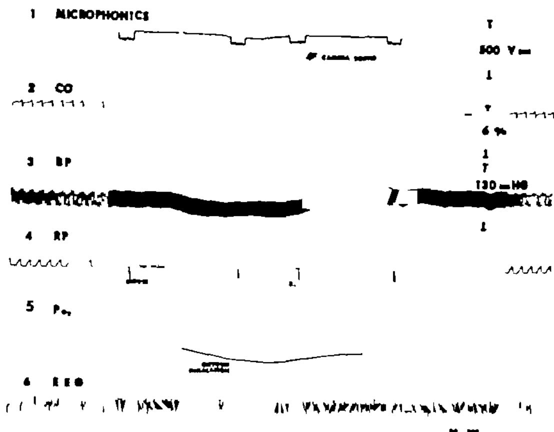


FIG 2. Th ffect of y temic hypo ia (5% oxyg n breathi g)

minutes Simultaneous measurement of blood flow velocity in the stria and of perilymph oxygen tension revealed no significant change Perfusion with higher concentration of cyanide (2 0 m*M*) produced a marked more sustained drop in microphonic output Unlike the 0 2 m*M* solutions, these higher concentrations of cyanide directly effect the performance of the polarographic electrodes by producing small farradic currents so that simultaneous values of perilymph oxygen tension could not be recorded by this method

A significant reduction in oxygen delivery to the cochlea to produce small reversible changes in perilymph oxygen tension and in microphonic output, was found possible by respiring the animal with 5% oxygen

Other effects of hypoxia are also recorded, such as flattening of the EEG increased cochlear blood flow drop in carotid blood pressure and in CO_2 of end expired air

Thus inhalation of 5% oxygen for 120 seconds results in a reversible drop in microphonic output from an initial RMS value of 340 μV to 200 μV Normal values are regained one minute after return to air breathing (20% oxygen) (Fig 2) Respiration with 5% oxygen for five minutes does not further increase the effect. At the same time strial blood flow is increased 37% Flow returns to normal five minutes after resumption of air breathing Perilymph oxygen tension drops to 26% of normal when the animal is respired with 5% oxygen and returns to normal level five minutes after resuming air breathing

is inactivated by cyanide. In the cochlea this is reflected in a greater reduction (50%) in function (microphonic output). This was reversible and the time needed to restore normal function was about the same as with 5% oxygen (7 minutes). On the other hand, oxygen tension in the perilymph did not drop to the same degree as with hypoxia alone. This may be due to a further decrease in oxygen utilization by the respiratory enzymes in cochlear cells. The additive effect of hypoxia and cyanide on the brain is reported by Levine & Stypulkowski (1959) who administered cyanide and ligated one carotid in the rat. They found more histological evidence of cerebral degeneration on the side of the carotid ligation.

ZUSAMMENFASSUNG

Die reversible Abnahme der Mikrophonenpotentiale wird durch endocochleare (Scala tympani) Durchströmung von 0,2 mM NaCN gefunden. 5%ige O_2-Inhalation kurz nach Durchströmung von NaCN verstärkt diese Abnahme der Mikrophonenpotentiale. Die Effekte von NaCN und Hypoxia auf Zytochromoxidase werden diskutiert.

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output in the cat was produced by Bornschein & Thalmann (1963) with 1 to 2 cc of 0.2% iodoacetate perfused into the perilymphatic space. A temporary reversible drop in output was also noted during infusion of fluids. This was attributed to increased intracochlear pressure.

The effects of cyanide prefusion on cerebral function are also of interest. Ward & Wheatley (1947) found that reversible obliteration of the EEG is produced by intravenous administration of 0.5 to 1.2 mg/kg NaCN. Russek *et al.* (1963) measured increases in cerebral blood flow with small doses of cyanide in cats and rabbits. They found no increase in the arteriovenous oxygen difference, suggesting that respiration of the brain was increased. On the other hand, Bloor *et al.* (1961) concluded that the intracarotid injection of cyanide (0.75 mg) in the monkey decreases respiration (oxygen utilization) of the brain since polarographic values for oxygen tension rose in the sagittal sinus, cerebral cortex, and cerebrospinal fluid.

B. Hypoxia. Normal cell function can be maintained with intracellular oxygen tensions of a few millimeters of mercury. With a microelectrode in muscle cells, Whalen & Nair (1966) obtained polarographic pO_2 values of about 3 mm Hg.

The inspired oxygen concentration that causes a decrease in oxygen utilization by the cell is designated by Chance *et al.* (1962) as the critical oxygen tension. He found that respiration of cells on the rat brain cortex is slightly (10%) reduced when the animal is respired with 8% oxygen. When 4% oxygen was used, cell respiration dropped by about half. Recent studies by Cohen *et al.* (1967) indicate that respiration with 7% oxygen affects aerobic cerebral metabolism in conscious man. Despite an increase in cerebral blood flow, the proportion of glucose undergoing complete oxidation was reduced and excess lactate appeared in the jugular venous blood. A critical oxygen tension may be considered for the cochlea. Wever *et al.* (1949) found that cochlear function (microphonic response) begins to decrease when the animal is respired with 4% oxygen mixtures. A similar value was found by Rice & Shinabarger (1961). We used brief (120 sec) periods of cochlear hypoxia (5% oxygen) and regularly produced a controlled, small reversible drop in cochlea function. Longer exposures to 5% oxygen (300 sec) did not further reduce the microphonic response. A measured increase in strial blood flow rate was due to relaxation of vascular smooth muscle and not to increase in perfusion pressure. The carotid blood pressure was reduced at this time while pulse pressure remained constant. General reduction in aerobic metabolism of the animal on 5% oxygen is indicated in the recorded drop in CO_2 tension of end expired air. With return to air breathing, a brief period of supernormal CO_2 production reflects a general increase in metabolic activity. While 5% oxygen mixtures produce a small drop in cochlear function, it has a strong effect on cerebral function (E.E.G. frontal lobe becomes flat).

C. *Hypoxia combined with cyanide perfusion.* Greater effects of hypoxia on cell respiration is noted when some of the terminal cytochrome oxidase

is a mere physical problem In this objective measuring technique, the cooperation of the subject is superfluous the corticofugal paths are not tested. Moreover the presence of an objective cortical reaction after acoustic stimulation does not necessarily correspond to the conscious perception of the administrated stimulus with a case of obvious hysterical deafness, which we were fortunate enough to follow accurately we always registered normal reactions with the cortical audiogram, where the psychoacoustically determined thresholds for pure tones remained 60 dB below normal and the speech discrimination was very poor for some three weeks. The subjec tive "restitutio ad integrum of audition that appeared after 1 month did not change the cortical audiogram in any way Many investigators proved the influence of the psychic condition of the subject (attention, indifference drowsiness, or sleep) on the obtained curves. The shape of the registered potential changes ultimately depends upon the technique During the first 50 σ after the beginning of the acoustic stimulation the myogenic potential of the sonomotor reflex (Bickford *et al* 1964 Cody *et al* 1964 *Mast* 1965) is registered. It originates in the neck muscles, the musculus temporalis, and the external ear muscles and is supposed to be connected with the functional condition of the vestibular apparatus. This potential can be derived in general from the skin covering the striate muscles. It increases with artificial increase of mechanical tension of the neighbouring muscles—especially of the neck muscles—and disappears almost completely with total muscle relaxation. In a recent publication Ruhm *et al* (1967) assume that this early component is also of cochlear origin.

The beginning of the proper slow evoked potential is somehow masked in the registration by the end of the myogenic potential It concerns a series of alternately vertex negative (V) and vertex positive (P) peaks that correspond with the V potential produced by acoustic, tactile, or visual stimuli (Davis, *et al* 1939) The term "V potential was introduced by Ban caud in 1953 t indicate that the most important reaction appears at the vertex. The different components of the V potential have a relatively con stant latency so that they distinguish themselves clearly from the EEG back-ground noise with an averaging technique. Davis (1965) assumes the following caracteristic latencies P 50–60 V 93–10 σ P_2 170–200 σ V_2 200 σ. In some of the curves these 4 peaks can clearly be distinguished but not always. Most constant is the descending slope of V to P_2.

METHOD

We tried to determine the influence of three stimulus parameters (ran d m on periodically administrated stimulus, intensity and frequency of the stimulus tone) on the obtained objective cortical reaction of subjects with a fully normal tonal audiogram. All experiments took place in two acoustically and electrically shielded rooms. In the first, the relaxed subject was lying on a seat with a headphone and electrodes applied to the head.

CORTICAL AUDIOMETRY IN NORMAL HEARING SUBJECTS

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Our present investigation deals with the influence of three stimulus parameters on slow evoked potentials derived from the intact scalp after acoustic stimulation (sine tones of 60 σ duration) in normal hearing subjects. Random application of the stimulus (50 times, every 1 to 3) is more efficient than a periodical stimulation (60 times, one stimulus every second) When stimulus intensity increases, the intensity of the reaction—mainly N_1-P_2—also increases, and the latency—mainly N and P_2—decreases provided that the 40 dB stimulation intensity is not exceeded The lower frequencies (250 500 1000 and 2000 c/s) give rise to a much clearer cortical reaction than the higher frequencies (4000 and 8000 c/s)

Auditory threshold determination in humans who do not possess the normal introspection possibilities (children or mental-deficients) or do not want to use them (simulators) has always been a very acute problem

The first technique of objective audiometry—the psychogalvanic reflex—was described by Féré in 1888 Experience has shown that his results are to be interpreted with reserve Psychic, visual and tactile stimuli also affect electric skin resistance and can thus cause the disappearance of the response to acoustic stimulation in background noise Moreover only lesions of the peripheral auditory apparatus, the auditory nerve the cochlear nuclei and the mesencephalon influence the acoustic psychogalvanic reflex

During recent years a technique has been discovered that deduces from the intact human scalp the evoked potentials these arise not only after acoustic but also after tactile and visual stimulation The cortical reaction has an approximate value of a few microvolts and disappears in the important electric background noise of the scalp (e g the EEG) and thus cannot be analysed by means of a simple oscillographic registration It can however be discovered with special averaging techniques by means of an average response computer because it is time locked to the beginning of the sensorial stimulus If the sensorial stimulus is repeated a few times, the accidental potential changes of the EEG eliminate each other so that the stimulus locked reaction becomes clearer on the zero line of the EEG

In all physiological measurements, three factors are to be taken into account the administrated stimulus, the subject, and the registration As in clinical tonal audiometry the exact determination of the acoustic stimulus

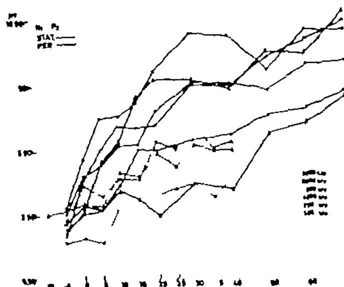


Fig. 3 Comparison of the reaction intensity of V_1-P (in μV) in the random (STAT) and the periodical (PER) stimulation series.

dl meter) a differential amplifier a Data Retrieval Computer and an X Y-plotter

The ground electrode was placed on the left ear lobe one differential electrode was placed 2 cm to the left of the vertex, in an interaural plane, and another on the right ear lobe These electrodes were connected to a low level amplifier type 122 Tektronix, set at a gain of 1000 with a band pass from 0.8 to 1000 cps. The amplified signal was connected without attenuation to the Data Retrieval Computer Model 7100 (Nuclear Chicago) The signals that were time locked to the stimulus were added, whereas the other signal were averaged. After each series of 50 random or 60 periodical stimuli, the contents of the memory of the Data Retrieval Computer were registered in analog form with an X Y-plotter model 7590 (AR(S) (Nuclear Chicago) From these graphs we read the extent of the reaction and converted them into an average potential (on the electrodes) for each stimulus.

In both series of measurements the same sound stimuli were used. A pure sine tone generated by a Bruel & Kjaer low frequency generator was connected without any amplitude change to a *four diode gate*. The gating signal was produced by a monostable multivibrator followed by two integrators, one influencing the rise time and the other the decay time. The time constants had been chosen such that no clicks were to be heard. The envelope of the gating signal is given in Fig. 1 *a* The duration up to 50% of the curve was 60 ms. In the periodical series the monostable multivibrator was triggered by the Data Retrieval Computer itself at the beginning of every analysis period. The analysis time was one second. In the random series separate trigger pulses were generated. They had random intervals

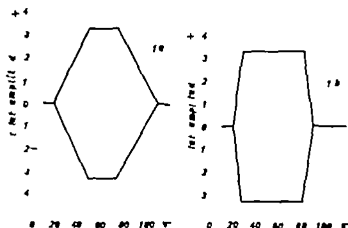
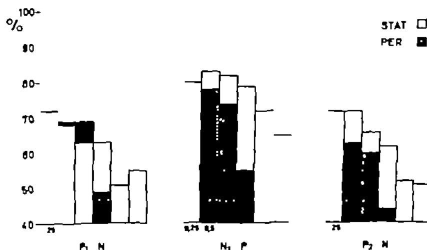


Fig. 1 Shape of the stimulus used.

In the second room were placed stimulation and registration apparatus. In a first periodical series we used sine waves (duration 60 σ) of 500 1000 and 2000 cps at – 10 – 5 0 5 10 15 20 25 30 35 and 40 dB sensation levels on 81 subjects (42 men and 39 women from ages 10 to 70) The acoustic stimulus was presented sixty times every second In the second—random—series we used sine waves (duration 60 σ) of 250 500 1000 2000 4000 and 8000 cps at – 5 0 5 10 15 20 30 40 50 60 and 70 dB sensation levels on 101 subjects (47 men and 54 women from ages 14 to 63) The acoustic stimulus was presented fifty times with random intervals from 1 to 3" In both experimental series the several frequency intensity combinations were used in a random order No more than 18 points were measured on a single subject Every measurement was repeated on 25 different subjects, in order to have statistically reliable results and to neutralize partially the considerable physiological dispersion

The apparatus used consisted of three electrodes (silver discs of 10 mm



F 2. Compariso f th mean pe ce t f po iti eacti (P N N P, P N) – 5 0, 5, 10 15, 20 30, a d 40 dB timul tion I te sity per frequ ncy (0 25, 0.5, 1 2, 4 8 kc) in th rand m (STAT) and the periodical (PER) stim l ti se ies

TABLE 1 *Statistical analysis of the results in the random stimulation series*

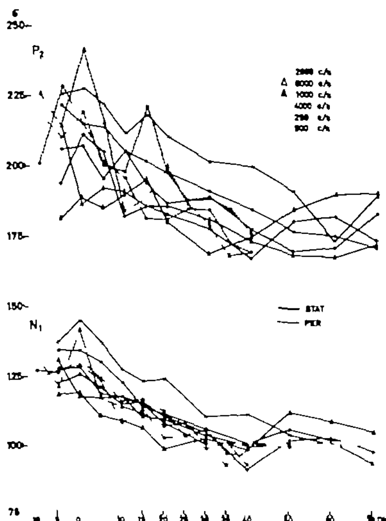
Val of *t* where the latency (P_1 N P_2 N_2) is statistically shorter on 40 dB than on 0, 10, and 20 dB stimulation intensity; value of *t* where the latency (P_1 N P_2 N) is statistically longer on 40 dB than on 70 dB stimulation intensity. Value of *t* where the reaction (P_1 N N–P P_2–N) is statistically greater on 40 dB than on 0, 10, and 20 dB stimulation intensity; value of *t* where the reaction (P_1 N N–P P N) is statistically smaller on 40 dB than on 70 dB stimulation intensity. Test 25 ears. In italics $P <$ 0.01. Ordinary type $0.01 < P$ 0.05.

	250 Hz, dB				500 Hz, dB				1000 Hz, dB			
40 dB	0	10	20	70	0	10	20	70	0	10	20	70
P_1	—	*13.05*	—	—	*17.34*	*4.46*	—	—	4.72	—	—	—
N_1	*16.11*	*12.71*	*8.61*	*14.8*	*41.02*	*10.41*	*7.70*	—	*20.16*	*7.80*	—	—
P_2	*20.40*	6.09	4.70	—	*22.66*	*11.38*	4.11	4.01	*18.82*	4.32	—	—
N_2	*8.44*	—	—	—	6.95	—	—	—	*12.23*	—	—	—
P_1-N_1	5.83	—	—	*10.04*	*11.40*	—	—	—	5.53	—	—	*10.72*
N_1-P_2	*17.21*	5.28	4.82	*11.13*	*20.24*	*4.47*	—	—	*30.44*	6.65	—	*11.10*
P_2-N_2	*10.99*	—	4.05	—	*8.41*	—	—	—	*16.37*	—	—	*8.18*

	2000 Hz, dB				4000 Hz, dB				8000 Hz, dB			
40 dB	0	10	20	70	0	10	20	70	0	10	20	70
P_1	*8.78*	—	—	—	—	—	—	—	—	—	—	—
N_1	*26.38*	*11.87*	—	—	*20.33*	8.48	—	—	—	—	—	—
P_2	*21.46*	*14.63*	—	—	*17.70*	5.31	—	—	—	—	—	—
N_2	*17.00*	—	—	—	*9.85*	4.21	—	—	—	—	—	—
P_1-N_1	—	—	—	5.60	—	—	—	6.48	—	—	—	*37.23*
N_1-P_2	*16.83*	*8.89*	—	—	*11.43*	5.10	—	4.19	—	—	—	*31.50*
P_2-N_2	*17.66*	—	—	—	5.01	—	—	—	—	—	—	*10.83*

more frequently in the random than in the periodical series. Moreover the cortical reaction in the random series is much more important than in the periodical series—this appears in Fig. 3, which shows the reaction intensity (μV) of N_1-P_2 as a function of the stimulus intensity for both the periodical and the random series. Our results confirm the statements of Keidel and collaborators, who have frequently drawn attention to the better reaction to random stimulation than to periodical stimulation. In our experiments this difference is most striking on 2000 cps. On the other hand, the latency has no relation to the way of administering the stimulus, periodical or random (Fig. 4).

Table I gives a statistical analysis of the results obtained from the random series. For each of the six frequencies analysed, it is calculated whether there is a significant difference between the latencies P_1, N_1, P_2, and N_2 on 40 dB and on 0, 10, 20 and 70 dB, and further between the intensities P_1-N_1, N_1-P_2, and P_2-N_2 on 40 dB and on 0, 10, 20 and 70 dB. Both the latency and the intensity of the reaction appear to be much more dependent upon the stimulus intensity on the low than on the high frequencies: thi



F g. 4 Comparison of th l t n y of \ and P (in σ) in the random (STAT) nd the periodi al (PER) timulation series

from 1 to 3″ These pulses triggered the stimulus generator and the Data Retrieval Computer The pulsed sine vawes thus produced were now further amplified up to a reference level measured by a vacuum tube voltmeter A manual control was provided to open the gate and give continuous sine waves for calibration Thereafter followed a calibrated attenuator and a power amplifier to which a headphone (Beyer DT 48) was connected

RESULT

Most investigators take the value of $N_1 P$ as a standard for the intensity of the reaction it is the highest and most frequent peak, as appears from Fig 2, which represents the average percentages of positive reactions of the three peaks on −5 0 5 10 15 20 30 and 40 dB for the different frequencies As each point was measured 25 times, we are dealing with average results of 200 (8 × 25) registrations From Fig 2 it appears also that the three peaks, with the minor exception of $P_1 N_1$ on 500 and 1000 cps, occur

normal. La stimulation acoustique à intervalles irréguliers (50 stimulations a ec un intervalle variable (1" à 3") est plus efficiente que la stimulation régulière (60 stimulation avec u intervalle de 1) L'intensité d la réaction (principale ment N_1-P_2) gmente et le temps de latence (principalement N et P_2 pour autant que les 40 db d'intensité stimulatoire e soient pas dépassés) diminue en fonction de l'intensité de la stimulation acoustique Les fréque ces importantes pour la compréhension de la parol humain (250 500 1000 et 2000 c/s) d nnent une réaction corticale beaucoup plus intense que les fréque ces aigues (4000 et 8000 c)

ZUSAMMENFASSUNG

Wir haben den Einfluss von drei Faktoren auf die durch akustische Stimulation (Sinustöne v n 60 s) hervorgerufene Hirnpotentiale beim normalen M n schen geprüft. Die akustische Reizung i unregelmässigen Intervallen (50 Stimuli mit einem unterschiedlichen Intervall von 1 bis 3 Sekunden) ist wirksamer als di regelmässige Reizung (60 Stimuli mit einem Int rvall von iner Sekund) Die I tensität der Reaktion (besonders N_1-P) nimmt zu, und di Latenzzeit (be sonders N und P sofern ei e Reizintensität von 40 dB nicht überschritten wird) nimmt in Abhängigkeit on der Intensität des kustischen Reizes ab. Die für den m nschlichen Sprachbereich wi htigen Frequenzen (250 500, 1000 und 2000 Hz) erzeugen eine viel stärkere kortikale Reaktion als die hohen Frequenzen (4000 und 8000 Hz)

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is clearly illustrated by the difference between the results on 250 and 8000 cps As to the latencies, we notice that on the 24 points examined for each peak there is a statistically significant difference 5 times for P_1 13 times for N_1 13 times for P_2, and 6 times for N_2. The latencies N_1 and P_2 appear to be the most stimulus intensity locked For those two peaks we notice a statistically significant difference 10 times between 10 and 40 dB and only twice between 40 and 70 dB for the 12 examined points above the 40 dB stimulus intensity the latency remains almost constant (Fig 4) Table 1 shows for $P_1 N_1$ 8 times, $N_1 P_2$ 15 times, and for $P_2 N_2$ 8 times a statistically difference on the 24 points examined for each peak The intensity of $N_1 P$ thus appears to be most closely related to the stimulus intensity

Besides the stimulus intensity the frequency of the stimulus has an influence on the reaction intensity *The best reaction is found in the speech* area, as appears from Fig 3 for $N_1 P_2$ on 4000 and 8000 cps the reaction is considerably lower than on 250 500 1000 and 2000 cps—the statistical analysis of the results completely confirms this statement

To avoid any doubt about the influence of the shape of the stimulus on the change in latency as a function of the intensity 25 control measurements have been carried out at 3 intensities 10 40 and 70 dB and at 6 frequencies 250 500 1000 2000 4000 and 8000 cps (450 points) All the measured latencies are about 12 σ smaller whereas the rise time of the stimulus has been decreased from 30 σ to 8 σ (Fig 1 *b*)

DISCUSSION AND CONCLUSIONS

As expected random stimulation gave a bigger reaction than periodical stimulation There was no other difference observable in the evoked potentials the latencies were the same and also the relation between the intensity of the stimulus and the amplitude of the potentials For that reason the major part of our work was done with random stimulation

A change of the latencies as a function of the stimulus intensity was also expected and was found only for intensities $\leqslant 40$ dB of the sensation level *and for the* N_1 *and* P_2 *peaks.* There was no change in latency for stimuli above 40 dB of the sensation level and the latencies of the P_1 and N_2 peaks seemed to remain almost constant.

Amongst the evoked potential peaks, the total amplitude $N_1 P_2$ was most closely related to the stimulus intensity and seemed to be most suitable to study the slow evoked potentials after acoustic stimulation

An unexpected result was the following although the sensation level was always taken as a reference for the acoustic stimuli, the amplitude of the evoked potentials was higher for frequencies in the speech area. A more intensive study of this phenomenon seems to be indicated

RÉSUMÉ

Nous étudions l'*influence de trois facteurs sur les* potentiels evoquées cérébraux dérivés après stimulation acoustique (tons sinusoidaux de 60) chez l'individu

normal. La stimulation acoustique à intervalles irréguliers (50 stimulation avec un intervalle variabl d à 3") est plus efficiente que la stimulation régulière (60 stimulations avec un intervalle d 1") L'inte sité de la réaction (principalement N_1P) augmente et le temps de latence (principalement N et P_2 pour autant que les 40 db d'intensité stimulatoire ne soient pas dépassés) diminue en fonction de l'intensité de la stimulation acoustique Les fréquences importantes pour la compréhension de la parole humaine (250 .00 1000 t 2000 c/s) don ent une réaction corticale beaucoup plus intense que les fréquences igues (4000 et 8000 c)

ZUSAMMENFASSUNG

Wir h ben den Einfluss von drei Faktoren auf die durch akustische Stimulation (Sinustöne von 60) hervorgerufen Hirnpotential beim normalen Men chen geprüft. Die akustische Reizung in unregelmässigen Intervallen (50 Stimuli mit einem unterschiedlichen Intervall von 1 bis 3 Sekunden) ist wirksamer als die regelmässige Reizung (60 Stimuli mit einem Intervall on einer Sekund) Die Intensität der Reaktion (besond rs N_1P) nimmt zu, und die Latenzzeit (besonders N und P sofern eine Reizi tensität von 40 dB nicht überschritten wird) nimmt in Abhängigkeit von der Intensität des akustische Reizes ab Die für den menschlichen Sprachbereich wichtigen Frequenzen (250, 500 1000 und 2000 Hz) erzeugen in viel stärkere kortikale Reaktion ls die hohen Frequen en (4000 nd 8000 Hz)

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THE DIAGNOSTIC SIGNIFICANCE OF SPONTANEOUS NYSTAGMUS AS OBSERVED IN THE ELECTRONYSTAGMOGRAPHIC EXAMINATION

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A study of spontaneous nystagmus in 121 normal subjects and 1601 patients suggests that an "idiopathic spontaneous nystagmus" is recorded with the subject's eyes closed in 20–25% of both the normal and patient population. This nystagmus is probably of no significance in the diagnosis of vertigo-producing disorders. It is of low intensity (usually below 6°/sec but occasionally in the 6–10°/sec range) and is more frequently directed to the left than to the right. Spontaneous nystagmus above 10°/sec is of diagnostic significance. It is probable that such a nystagmus will be of peripheral origin and directed away from the side of the peripheral lesion.

Visual fixation is a powerful suppressant of vestibular nystagmus, and therefore recording with electronystagmography (ENG) in the absence of visual fixation very often permits observation of a spontaneous nystagmus which cannot be seen with the patient's eyes open in the light (Aschan *et al.* 1956).

However, in the literature on electronystagmography there is considerable controversy as to the diagnostic significance of spontaneous nystagmus once it is recorded. In contrast, the older literature dealing with spontaneous nystagmus present with eyes open is generally in *agreement that* spontaneous nystagmus is always pathological and, when caused by an acute peripheral vestibular deficit, is directed away from the side of the deficit (Fischer 1956, Alpers, 1958).

A systematic study of spontaneous nystagmus as recorded electronystagmographically with a goal of redefining the diagnostic significance of this finding, would appear to be desirable. The results of such a study are presented. Emphasis is placed on the significance of the intensity and the direction of the recorded spontaneous nystagmus.

In this report we will consider only "vestibular" spontaneous nystagmus,
direction of the recorded spontaneous nystagmus.
pressed by visual fixation. We have not considered "central" or "ocular" nystagmus (cf. Dix *et al.* 1963) defined as a nystagmus which is enhanced or unchanged in intensity by visual fixation or which is of different form or direction with eyes closed in comparison to eyes open.

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MATERIAL AND METHOD

ENG Examination Procedure

General

All of the normal subjects and patients included in this study were given electronystagmographic examinations at The Methodist Hospital. This examination consisted of a 20° calibration, a test for gaze nystagmus, a position test according to the procedure suggested by Nylén (1939) and later by Aschan *et al.* (1956), an optokinetic test, and a Fitzgerald-Hallpike caloric test. The caloric responses were quantiated by determining maximum eye speed, and unilateral weakness and directional preponderance were calculated as a percentage of the total response intensity. Additional details of the test procedure are published elsewhere (Coats, 1965, 1966).

Recording parameters

Eye movements were recorded on an Offner type R Dynograph with an input time constant of one second and a gain of 100 µV/cm pen deflection. At this gain, eye-movement calibrations ranged from 0.5 to 2°/mm.

Criteria for determining the presence or absence of spontaneous nystagmus

In order to be considered significant, a spontaneous nystagmus was required to be present, uninterrupted, for at least 30 seconds while the patient was supine during the position test. Also it had to be present in the caloric test position before the start of the first irrigation.

Measurement of spontaneous nystagmus intensity

Spontaneous-nystagmus intensity was determined by averaging the slow component speeds of at least 5 beats judged to be representative of the average spontaneous-nystagmus intensity. All records were measured by a technician who had no knowledge of the purpose of the study.

Normal Subjects

ENG examinations were given to 121 normal subjects (42 females and 79 males) selected from medical, dental and nursing school students and medical center technical personnel. Ages ranged from 18 to 30 years. All subjects had normal caloric tests and none complained of vertigo or hearing loss.

Patients

Records of 1783 consecutive patients seen at the ENG laboratory in The Methodist Hospital were reviewed. 182 patients were excluded from the study for the reasons listed in Table 1.

The remaining 1601 patients were divided into three major groups, as shown in Table 1. The criteria for each of these groups were as follows:

TABLE 1 *Classification of patients*

Group			
I	Unilateral weakness		364
II	Other noncentral (D.P. S.N., pos., normal)		1106
III	Evidence of central abnormality		
	A. Evidence other than OPK-gaze NYS		60
	B. Only indication is OPK or gaze NYS		14
	C. CNS abnormality with unilateral weakness		36
Total included in study			1601
Patients excluded from study			
Bilateral caloric weakness			42
Not classifiable			
No clinical information		14	
Incomplete caloric test		81	
Mastoidectomy		12	
Total not classifiable			107
Poor records			10
Ocular or "central" spontaneous nystagmus			21
Other ocular abnormality			2
Total excluded from study			182
Total — all patients			1 83

Group I Unilateral weakness

In this category were placed all patients with caloric responses from one labyrinth which differed from the responses from the opposite labyrinth by more than 20% of the total response. Presumably most patients in this group had unilateral peripheral vestibular lesions.

Group II All other noncentral

In this category were placed all of the remaining patients whose ENG and neurological findings did not permit diagnosis of a central nervous system abnormality. Undoubtedly this group includes a large variety of non vestibular causes of dizziness as well as abnormalities of the peripheral vestibular apparatus which do not produce caloric unilateral weakness (e.g., lesions involving the otolith organs but sparing the semicircular canals).

Group III Evidence of central nervous system abnormality

All patients with one or more of the following findings were placed in this group (1) an optokinetic asymmetry and/or unilateral gaze nystagmus not attributable to a spontaneous nystagmus (Coats, 1968) (2) bilateral gaze nystagmus with eye deviations of 20° from the midline (3) caloric nystagmus intensity with eyes open and fixed equal to or greater than

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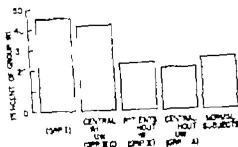


Fig. 1

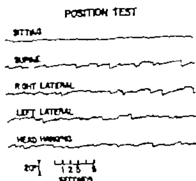


Fig. 2

Fig. 1 Incidence of pontaneous nystagmus in the patient groups listed in Tabl I (group designati ns show I parentheses) and in 121 ormal subjects.

Fig. 2. Example f spontaneou ystagmus I normal subject. The subject was 22 year-old right-handed male medical student who had had at fly our 2 weeks before the xamination. H had had no ther illness, major or minor for t least 6 months nd had never had ertigo-product g illness. There was histi ry f ingestion f lcohol or other drugs xcept for 2 cups of coffee per day The subject did not moke The tympa ic membranes were lear Audiometric and caloric test result were ormal. B th eyes, low intensity left be ting spontaneou ystagmus was present in ll but the itti g position

Int nsity of spontaneous nystagmus

Frequency distribution of spontaneous-nystagmus intensity in each of the subject groups are shown in Figs. 3 and 4 In all groups, the most frequently occurring intensity was 3–5 /sec (the modal value) However the unilateral-weakness group differed from the other groups in that there were many more patients in the higher intensity ranges. To facilitate further study of this difference spontaneous-nystagmus intensity was divided into three ranges "low" (0 1–5.9 /sec) "moderate" (6.0–9 9 /sec) and "high" (greater than 9.9 /sec) Two (1 6%) of the "normal" subjects had a spon taneou nystagmus in the high intensity range This is in essential agreement with the results of Bos *et al* (1963) who found that 3% of their nor mal subjects had spontaneous nystagmus with an intensity greater than 7 0 /sec

Fig. 5 shows a comparison of the incidence of spontaneous nystagmus in th three intensity ranges. In all three ranges, the incidence of spontane ous nystagmus among patients with unilateral weakness is higher than am ng patient without unilat ral weakness. However this difference be comes progressively greater as spontaneous-nystagmus intensity increases. The percentages in the three groups without unilateral weakness (central, noncentral, and normal) do not differ significantly

Direction of spontaneous nystagmus

The relative incidence of left nd right-beating spontaneous nystagmus in the different groups is sh wn in Fig 6 It is apparen that in the low

caloric nystagmus intensity with eyes closed ("failure of fixation suppression" of caloric nystagmus) (4) definite clinical evidence of a central nervous system abnormality

Group III was subdivided as shown in Table 1 Since one of the goals of this study was the comparison of the incidence and intensity of spontaneous nystagmus in the presence of central and peripheral pathology those patients with both central and peripheral pathology (most had mass lesions in the cerebellopontine angle) were grouped separately (Group III C) Fourteen patients in whom the only indication of a central abnormality was an optokinetic asymmetry or gaze nystagmus not attributable to a spontaneous nystagmus (Group III B) were excluded from consideration in order to avoid the use of a criterion based on spontaneous nystagmus to select a group of patients in whom the incidence and intensity of spontaneous nystagmus was to be studied

The 69 patients in Group III A constitute the CNS abnormality group of primary interest in this study In 54 of these patients, the presence of CNS pathology was confirmed by evidence other than that provided by the ENG examination Patients in this group had central lesions of widely varying location and type

RESULT

Incidence of spontaneous nystagmus

The percentage of subjects in each group who had a spontaneous nystagmus in the supine position is shown in Fig 1 Almost half of the patients with caloric unilateral weakness had a spontaneous nystagmus, compared to only 20–25% in the other groups

The incidence of spontaneous nystagmus in normals is in essential agreement with the results of Bos *et al* (1963) who found nystagmus in one or more positions in 24% of their normal subjects, and of Bergstedt (1961) who found positional nystagmus in 9 of 26 normal subjects However it is not in agreement with the statements of other investigators (e.g Stahle, 1958) that a spontaneous nystagmus observed with eyes closed is always an abnormal finding A typical example of a spontaneous nystagmus observed in a normal subject is shown in Fig 2

It should be emphasized that the recordings on which this report is based were obtained with eyes closed, and not with eyes open in darkness. Dix *et al* (1963) reported that spontaneous nystagmus of central or ocular origin may differ in form or direction when the eyes are closed in comparison to eyes open in darkness. Preliminary results of a study of the nature of spontaneous nystagmus in normals show that opening the eyes in darkness tends to reduce the intensity of the spontaneous nystagmus but never alters its form or direction The preliminary study also shows that spontaneous nystagmus in normals adds to caloric nystagmus to produce directional preponderance just as it does in patients (Coats, 1966)

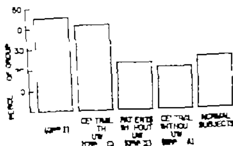


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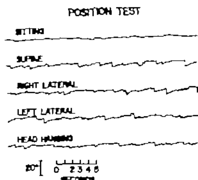


Fig. 2.

Fig. 1. Incidence of spontaneous nystagmus in the patient groups listed in Table 1 (group designations shown in parentheses) and in 121 normal subjects.

Fig. 2. Example of spontaneous nystagmus in a normal subject. The subject was 22-year-old right-handed male medical student who had had stuffy nose 2 weeks before the examination. He had had no other illness, major or minor, for at least 6 months and had never had vertigo-producing illness. There was no history of ingestion of alcohol or other drugs except for 2 cups of coffee per day. The subject did not smoke. The tympanic membranes were clear. Audiometric and caloric tests results were normal. With eyes closed low-intensity left-beating spontaneous nystagmus was present in all but the sitting position.

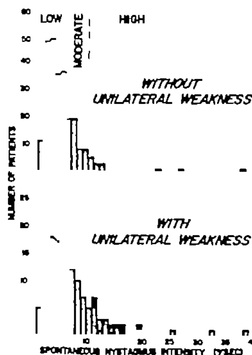
Intensity of spontaneous nystagmus

Frequency distributions of spontaneous-nystagmus intensity in each of the subject groups are shown in Figs. 3 and 4. In all groups, the most frequently occurring intensity was 3.5°/sec (the modal value). However the unilateral-weakness group differed from the other groups in that there were many more patients in the higher intensity ranges. To facilitate further study of this difference, spontaneous-nystagmus intensity was divided into three ranges: "low" (0.1–5.9°/sec), "moderate" (6.0–9.9°/sec) and "high" (greater than 9.9°/sec). Two (1.6%) of the "normal" subjects had a spontaneous nystagmus in the high-intensity range. This is in essential agreement with the results of Bos *et al.* (1963) who found that 3% of their normal subjects had spontaneous nystagmus with an intensity greater than 7.0°/sec.

Fig. 5 shows a comparison of the incidence of spontaneous nystagmus in the three intensity ranges. In all three ranges, the incidence of spontaneous nystagmus among patients with unilateral weakness is higher than among patients without unilateral weakness. However this difference becomes progressively greater as spontaneous-nystagmus intensity increases. The percentages in the three groups without unilateral weakness (central noncentral and normal) do not differ significantly.

Direction of spontaneous nystagmus

The relative incidence of left and right-beating spontaneous nystagmus in the different groups is shown in Fig. 6. It is apparent that in the low



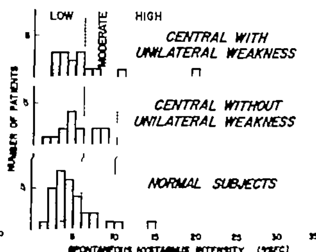
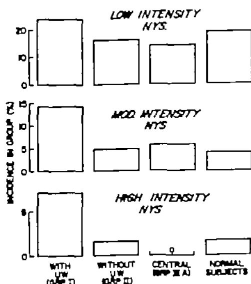
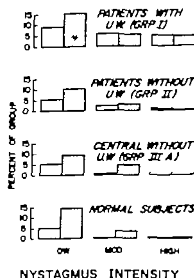


Fig 3 Fig 4

Fig 3 Frequency distribution of spontaneon nyst gmus in n ncentral patients with (Group I) and without (Group II) caloric unil t ral weakness. Because of the much large number of pati nts with ut unilateral weakness, the scale i terval n the ertical xis was d ubled

Fig. 4 Frequency distributi n of spontaneous-nystagmu int nsity i central patients (Group III A nd C) d n m l subjects.





F 5 Fi 6

Fig 5 In id nc of pont neou ystagmu f l w mod rat d high i t n ty T f ilit te mpari th rtic l scal w dj sted t m k th pl t f i id nce l the unil t ral weaknes g up of equal h ight

Fig 6 Incidence f right (st ppl d bars) d l ft be ti g (cro -hat h d ba) po t neo s ; tagmu t th th ee t nsity l) } pati nt gro ps d normal ubjects.

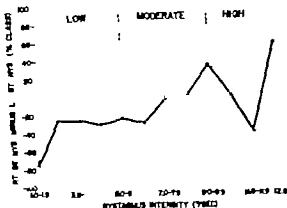
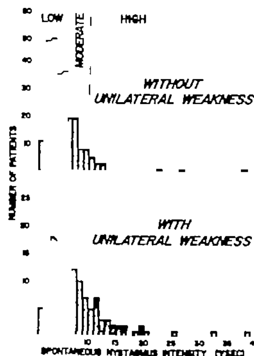


FIG. 7. Effect of intensity on predominance of left over right-beating spontaneous nystagmus. All noncentral patients (Groups I and II) with spontaneous nystagmus were separated into intensity classes at 1°/sec intervals (*horizontal coordinate*). The values plotted on the vertical scale were obtained by expressing the difference between numbers of patients with right and left-beating nystagmus as a percentage of all patients falling into the intensity interval. Negative values mean predominance of left over right beating spontaneous nystagmus. Large fluctuations occur at the higher intensity levels because relatively small numbers of patients fell into these intensity classes.

intensity range, left-beating spontaneous nystagmus predominates in all groups. In the moderate-intensity range, left beating spontaneous nystagmus predominates in the central and normal groups but not in the unilateral weakness and non-unilateral-weakness patient groups. In the high-intensity range the incidence of right and left beating spontaneous nystagmus is about equal in all groups.

In Fig. 7 the relative predominance of right or left beating nystagmus over each 1° intensity increment in Groups I and II is plotted against intensity. In every class below 7.0°/sec, left beating nystagmus predominates; above 7.9°/sec, incidence of left and right beating-spontaneous nystagmus is essentially equal. The limits of the moderate-intensity range were chosen so that the intensity at which right versus left incidence became equal (7.0–7.9°/sec) would lie equally between them. It is of interest that the intensity at which right-left incidence becomes equal is close to the 6–7°/sec "threshold of pathological nystagmus" proposed by Bos *et al* (1963).

In both incidence and predominance of direction to the left, low intensity spontaneous nystagmus in the unilateral weakness group closely resembles low-intensity spontaneous nystagmus in the three non-unilateral weakness groups. Therefore, it would seem probable that, frequently, low intensity spontaneous nystagmus, when associated with a unilateral weakness, is not related to the unilateral weakness. This was supported by a comparison of the direction of spontaneous nystagmus relative to the side of the unilateral weakness in the three intensity ranges. The results are shown in



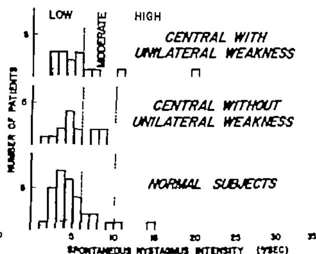
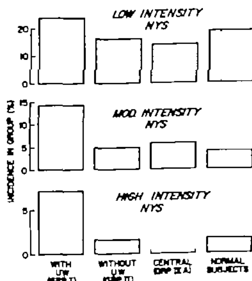
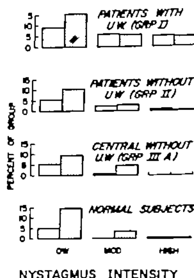


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Fig 3 Frequency distribution of spontaneous nystagmu in noncentral pati nt with (Group I) and with ut (Group II) cal ric unil t ral weakness Because of th much larg r number of patients with ut unilateral weakness, the scale i t rval on th v rtical axis was d ubled

Fig 4 Frequ ncy di tribution of pontaneou ny tagmus intensity in central patie t (Gro p III A and C) a d normal subjects.





F o F 0

Fig 5 Incid nc of spo t eou stagmu of l w moderat a d h gh int it To facilitat compari th rtic l scal w dj sted t m k th pl t f l d ce i the u il teral weak g up of equal h ight

F o 6 I d nc of right (t ppl d bars) nd l ft beati g (w-h t h d bars) po t neou n st gmu t the three nt ity l els in pati t groups nd orm l bjects.

Direction of spontaneous nystagmus in patients with caloric unilateral weakness

Stahle (1958) reported that the spontaneous nystagmus was directed away from the affected side in 11 of 13 patients with both "diminished [caloric] sensitivity and spontaneous nystagmus in the caloric test position. In contrast, Jongkees & Philipszoon (1964) reported that the direction of spontaneous nystagmus is poorly correlated with the side of a peripheral vestibular lesion and, therefore concluded that the direction of spontaneous nystagmus is of no significance. The present study shows that the results of Jongkees & Philipszoon (1964) are obtained only if the intensity of the nystagmus is not considered. If one considers only spontaneous nystagmus of moderate and high intensity a good relationship between the direction of the spontaneous nystagmus and the side of the peripheral lesion is found.

Spontaneous nystagmus in patients with central nervous system pathology

It is of particular interest that the spontaneous nystagmus found in the central group could not in any way be distinguished from idiopathic spontaneous nystagmus occurring in the normal group (it occurred in less than 25% of the patients, was predominantly left-beating, and was exclusively of low or moderate intensity). Because of the broad definition of "central" this result does not exclude the possibility that specific lesions within the central nervous system might produce a vestibular type spontaneous nystagmus. However, that no such lesion was demonstrated among 1601 patients would suggest that such a lesion is uncommon. *Therefore* a spontaneous nystagmus above 7.0°/sec is probably an indication of a peripheral vestibular lesion.

It will be recalled that we have excluded from consideration nystagmus which, either with eyes open or closed, did not have a saw-toothed form or which was not suppressed by visual fixation. In our experience central lesions which are generally thought of as producing spontaneous nystagmus (e.g., lesions of the flocculonodular lobe of the cerebellum and multiple sclerosis involving the brainstem) produce nystagmus with one of these characteristics.

ZUSAMMENFASSUNG

Die Untersuchung eines spontanen Nystagmus bei 121 gesunden Personen und 1601 Patienten ergibt, dass sich bei 20–25% der sowohl gesunden Personen als auch der Patienten ein idiopathischer spontaner Nystagmus bei geschlossenen Augen zeigt. Der Nystagmus ist wahrscheinlich bei der Diagnose schwindelerzeugender Störungen von kleiner Bedeutung. Er ist von geringer Intensität (gewöhnlich unter 6°/Sek., manchmal jedoch in der 6–10°/Sek. Zone) und geht häufiger nach links als nach rechts. Spontaner Nystagmus über 10°/Sek. ist von diagnostischer Bedeutung. Es ist sehr wahrscheinlich, dass solch ein Nystagmus von äusserlichem Ursprung ist und von der Seite der äusseren Läsion weggeführt.

TABLE 2 *Direction of spontaneous nystagmus relative to side of caloric unilateral weakness (Group I with spontaneous nystagmus)*

Nystagmus intensity	Direction relative to side of U W	Number of patients	% all patients in intensity class
Low	Away	52	59 1
	Toward	36	
Moderate	Away	41	87.2
	Toward	6	
High	Away	25	83.3
	Toward	5	

Table 2 Low intensity nystagmus was directed away from the side of the weakness (the expected direction) in only 59% of the Group I patients with spontaneous nystagmus However in the moderate and high intensity ranges, this increased to more than 80%

DISCUSSION

Definition of idiopathic spontaneous nystagmus

We have found a spontaneous nystagmus in a rather large percentage of subjects presenting themselves as normal It seems highly unlikely that this nystagmus is of significance in the diagnosis of vertigo-producing disorders However because of the extreme sensitivity achieved by recording nystagmus in the absence of visual fixation and the impossibility of excluding minor abnormalities from a group of "normal" individuals, we would hesitate to call this a "normal" spontaneous nystagmus Therefore, the term idiopathic spontaneous nystagmus" used by Lansberg (1962) *is preferred*

On the basis of this survey the following characteristics of idiopathic spontaneous nystagmus can be defined (1) It is present with eyes closed in about 25% of people with no evident neuro-otological abnormality (2) Its speed of slow component is usually less than 6 /sec but may be as high as 9–10 /sec (3) It usually beats to the left but may be right beating

Idiopathic spontaneous nystagmus in patients

All of the results of this study support the hypothesis that idiopathic spontaneous nystagmus occurred in patients referred to the ENG laboratory as frequently as it occurred in the normal subjects This hypothesis would explain the predominance of left beating spontaneous nystagmus in the low intensity range in all subjects, regardless of clinical classification It would also explain the relatively small increase in incidence of low intensity spontaneous nystagmus among patients with unilateral weakness, and also the poor correlation between side of unilateral weakness and direction of low intensity spontaneous nystagmus in this group

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CALORIC TESTS IN PASSIVE VASCULAR CONGESTION OF THE LABYRINTH

An Electronystagmographic Study

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From the Department of Otolaryngology Postgraduate Institute of Medical Education and Research Chandigarh India

In order to evaluate the effect of passive venous congestion of the labyrinth on caloric induced nystagmus, thirty normal subjects were given caloric tests in the "head up" and "head down" positions. The *horizontal semicircular canal was kept in a vertical position* with the ampullated end upward when the head was in the down position and venous congestion was produced. The resulting nystagmus was recorded by an electronystagmograph. A comparison of the response of the labyrinth in the "head up" and "head down" positions reveals that the response is diminished in the "head down" position more so with cold irrigation. Thus it has been shown that passive venous congestion produces an effect on caloric induced nystagmus.

The response of the labyrinth to caloric stimulation is known to be influenced by various factors, e.g. position and posture of the subject, temperature of the water used for irrigation, central inhibition and repetitive caloric stimulation, amongst others.

McNally *et al* (1948) performed caloric tests in supine and prone (face down) positions and found the response to be greater in the supine position than in the prone position in a majority of the cases.

Mehra & Moudgil (1967) observed that response to hot and cold caloric stimulation in the supine position is very significantly higher than in the prone position. However they kept the head below the heart level and used nystagmographic recording for the study. They put forward the possibility of a passive venous congestion of the labyrinth affecting the caloric response.

Jongkees (1948) studied vascular influence on caloric response. He observed that vasodilation of blood vessels had an effect on the duration of calorically induced nystagmus, and also that such effect appeared slight during hot caloric stimulation and strong during cold caloric stimulation.

The aim of the present study was to evaluate the influence of passive vascular congestion of the labyrinth on the caloric responses. Vascular congestion was produced by raising the foot end of the table so as to bring the subject's ear below the heart level while still maintaining the lateral

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TABLE 1

		Cold caloric stimulation				Hot caloric stimulation			
		LCU*	LCD	RCU	RCD	LHU	LHD	RHU	RHD
LP	Mean	19.46	20.83	19.28	21.35	23.41	26.00	24.35	27.88
		4.9	5.2	4.5	6.0	4.6	8.2	4.4	8.3
TD	Mean	182.00	160.80	1 6.98	157.03	161.73	144.93	155.10	144.43
	S.D.	28.6	34.1	35.4	31.2	26.5	36.4	23.2	19.7
TB	Mean	225.03	173.10	222.46	169.30	147.00	127.70	137.06	129.30
	S.D.	60.0	57.0	65.7	63.6	40.1	45.8	38.3	53.2
TA	Mean	1616.13	1199.86	1635.43	1131.16	861.73	718.23	821.40	875.30
	S.D.	677.1	699.4	876.2	702.9	383.6	420.6	454.2	290.2
MSS	Mean	16.99	13.81	17.82	13.30	10.28	9.41	9.58	9.32
	S.D.	4.7	6.3	7.0	5.6	4.0	4.4	4.2	4.8

LCU = Left cold up, etc.

Analysis of data

The following observations of the records were made Latent period of onset of nystagmus (LP) Total duration of nystagmus (TD) Total number of beats (TB) Total amplitude (TA) and Maximum speed of slow component (MSS) The mean and standard deviation of various indices of the nystagmus of both series of tests is given in Table 1

The latent period and maximum speed of the slow component show very little variation of values. The total amplitude, total number of beats, and total duration show the maximum variation.

The statistical analysis of the results of the first irrigation with the

TABLE 2. Comparison between the cold and hot responses in the "up" position

Significant values of "t" at 0.05 probability 2.00 and at 0.01 probability 2.68.

		LCU RCU	LHU RHU	"t" value
LP	Mean	38.1	47.76	5.3
	S.D.	3.6	6.4	
TD	Mean	339.98	309.83	3.7
	S.D.	43.5	31.4	
TB	Mean	417.49	251.66	8.7
	S.D.	83.6	60.6	
TA	Mean	3231.56	1696.13	6.9
	S.D.	1107.1	501.6	
MSS	Mean	31.81	18.36	8.0
	S.D.	8.4	5.8	



Fig. 1

semicircular canal in a vertical position Engorgement of the neck veins was taken as the indicator of vascular congestion in the labyrinth

Alternate hot and cold caloric tests according to the Fitzgerald & Hallpike technique (1942) were performed on thirty young healthy adults in both the normal supine position and the head low supine position (Figs. 1 and 2) The resulting nystagmus was recorded on an electronystagmograph (Galileo type) This had an AC type preamplifier with a long time constant of 4.5 seconds The test was performed in a semi-darkened sound treated room After the application of bitemporal pure silver electrodes, calibration was performed by asking the subject to look at alternating light sublined at a 10-degree angle over the eyes It was repeated after the test After each stimulation a rest period of 10 minutes was given

Caloric tests were done in the following order (1) Left cold up, (2) right cold up (3) left cold down (4) right cold down (5) left hot up (6) right hot up (7) left hot down (8) right hot down

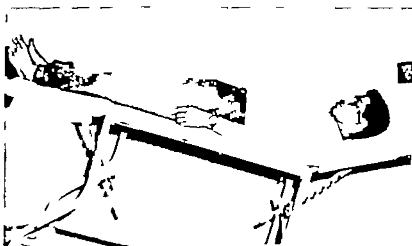


Fig. 2.

TABLE 5 *Comparison of the total "up" position with the "down" of cold and hot irrigation of the right and left ears*

Significant alue of "t" at 0.05 probability = 2.00 and at 0.01 probability = 2.88.

		LCU + RCU + LHU + RHU	LCD + RCD + LHD + RHD	"t" lues
LP	Mea	86.50	96.46	3 3
	S.D	9.3	13.7	
TD	Mean	689.81	604.19	3.6
	S.D	57 1	77.9	
TH	Mean	732.15	601.30	1.7
	S.D.	102.0	111.6	
TA	Mean	4937.89	4953.85	3.2
	S.	1257 6	1115.7	
MSS	Mean	51 67	46.07	2.7
	S.	10.3	10.8	

hot and cold irrigation statistical analysis of data was done and the following "t values resulted The results show that, except for the latent period, there has been a significant decrease of all the values of nystagmus in the down" position with cold irrigation, whereas it is not significant with hot irrigation

Comparison of the total up" responses of the right and left ears with the total down position of the right and left ears is given in Table 4 This table again shows that there is a diminished response in the "down" position

The total up position and total down position are compared in Table The result again shows that there is a greater diminution of response in the down position than that in the "up position Thus it can be summarised that pa ive venous congestion of the labyrinth may be one of the factors suppres ing the response Somehow this effect is more marked with cold stimulation than with hot stimulation Jongkees (1948) compared the result of cal ric tests in the usual position with those of the "upside down" position. H observed that duration of the nystagmus was shorter in the upside down than the u ual position He has also noted that the effect of vasodilatation of the labyrinth produced with amyl nitrite was less m rked with hot cal ric stimulation than with cold caloric stimulation. Our finding also seem to correspond with J ngkees Mehra & Moudgil 1967) also n ted a reduction of response in the "face down" position

It i postul ted from thi study that passive venous congestion of the l byrinth produces a diminl hed response t cold caloric stimulation. It is the intention of the authors to pursue this investigation on patients with raised jugular venou pressure

TABLE 3 *Comparison of 't' values between the cold and hot responses in the up and down positions*

Significant value of "t" at 0 05 probability – 2.00 and at 0 01 probability – 2.68.

	LCU + LCD	LHU + LHD	RCU + RCD	RHU + RHD
LP	1	1.84	1.7	2.0
TD	2.00	1 20	2.31	1.9
TB	3 62	1.62	3.2	1
TA	2.34	1 20	2.4	1
MSS	2.20	1	2.6	1

second irrigation and comparison of right and left ear sensitivity do not show any significant difference Lidvall (1962) had observed from his studies of repeated caloric stimulation that response decline to repeated stimuli is more pronounced with identical stimuli, but was of the opinion that transfer of habituation probably does not cause distortion of routine caloric tests

To note whether hot and cold stimulations are equal the total cold irrigation response in the "up" position is compared with the total hot irrigation in the "up position in Table 2 The result indicates that there is a marked reduction of all values of nystagmus except in the latent period responses of hot irrigation Thus hot stimulation is less effective than cold. Similar findings have been noted by McNally *et al* (1948) and Mehra (1964) However Aschan *et al* (1956) and Stahle (1956) have observed no difference

To compare individually each ear for the 'up and "down positions for

TABLE 4 *Comparison of responses of the "up position of the left and right ears with those of the down position in the same ears*

Significant valu of t at 0 05 probability – 2.00 and t 0 01 probability – 2.68.

		Left ear		t value	Right ea		t value
		LCU + LHU	LCD + LHD		RCU + RHU	RCD + RHD	
LP	Mean	43.63	49.23	2.2	86.50	96.46	3.3
	S.D	9 7	9 7		9.3	13.7	
TD	Mean	332 08	301 46	2.2	669.81	606.19	3.6
	S.D	68 6	36.9		57 1	77 9	
TB	Mean	360.12	298.50	3 0	732.15	601.30	4
	S.D	73 1	83.2		102.0	111 6	
TA	Mean	2456.83	2005 46	2.2	4937 69	3953.55	3.2
	S.D	816.1	760.4		1257 6	1115.7	
MSS	Mean	27 40	22.82	2.3	54.67	56.07	2.7
	S.D	7 7	7.5		10.3	10.8	

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DIE RICHTCHARAKTERISTIK PRIMÄRER AFFERENZEN DES OTOLITHENORGANS BEI INTAKTER EFFERENTER INNERVATION

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Meerschweinchen konnten auf einem kardanischen Tisch um alle drei Achsen des Raumes gekippt werden. Von primären Fasern des Nervus vestibularis wurden Aktionspotentiale abgeleitet. Auch bei intakter efferenter Innervation zeigen die primären Fasern eine Funktionsweise, wie sie von Löwenstein und Roberts am isolierten Rochenlabyrinth gefunden wurde. Die primären Fasern, die vermutlich benachbarte und daher ähnlich orientierte Rezeptorzellen innervieren, besitzen eine eindimensionale Richtcharakteristik. Daher müssen wenigstens drei verschiedene Rezeptortypen existieren, damit der Organismus eine umkehrbar eindeutige Information über die Lage im Raum gewinnen kann. Tatsächlich konnten verschiedene Rezeptortypen gefunden werden, die den von Spoendlin nachgewiesenen verschiedenen Orientierungsrichtungen der Macula-Rezeptoren entsprechen. Die Übereinstimmung der Funktionsweise primärer afferenter Fasern sowohl bei intakter als auch bei ausgeschalteter efferenter Innervation wirft die Frage der funktionellen Bedeutung der efferenten Innervation auf.

Arbeitsdiagramme von primären Maculaafferenzen wurden bereits 1950 von Löwenstein & Roberts (1950) veröffentlicht. Die Befunde wurden am isolierten Rochenlabyrinth gewonnen. Sie zeigen, daß in den primären Fasern eine Spontanaktivität von außerordentlicher Regelmäßigkeit besteht. Auf diese Spontanaktivität wird die Information über die Lage des Tieres im Raum durch Änderung der Entladungsrate aufmoduliert. Die Regelmäßigkeit der Entladungen bleibt dabei bestehen. Die Autoren beschreiben ferner noch sogenannte „out of position" Neurone und die „into level" Neurone. Diese Neurone zeigen bei Normalposition des Tieres minimale bzw. maximale Aktivität. Bei Kippung in jeder beliebigen Richtung nimmt die Aktivität zu bzw. ab.

Das Sinnesepithel der Vestibularorgane besteht aus sekundären Sinneszellen. Außer der afferenten Innervation dieser Zellen wurde auch eine efferente Innervation nachgewiesen. Diese Kenntnis verdanken wir elektronenoptischen Untersuchungen von Wersäll (1956) sowie Engström & Wersäll (1958), histologischen Arbeiten von Rasmussen & Gacek (1958), von Gacek (1960) und von Rossi & Cortesina (1962). Ferner liegen neuro-

Mit Unterstützung der Deutschen Forschungsgemeinschaft Gr 161/6 und Gr 161/8

ZUSAMMENFASSUNG

Um die Wirkung der passiv giftigen Stauung des Labyrinths auf den durch Kalorien herbeigeführten Nystagmus zu berechnen wurden bei dreissig normalen Subjekten kalorische Versuche bei hochgehaltenem und gesenktem Kopf durchgeführt Der horizontale halbzirkulare Kanal wurde in vertikaler Stellung — mit aufrechter Ampulle — gehalten wenn der Kopf in gesenkter Stellung war und giftige Stauungen wurden bewirkt Der erzielte Nystagmus wurde durch den Elektronystagmograph verzeichnet Ein Vergleich der Reaktionen des Labyrinths bei hochgehaltenem oder gesenktem Kopf hat gezeigt, dass die Wirkung bei gesenktem Kopf geringer ist um so mehr bei kalter Irrigation Somit zeigte sich, dass passiv giftige Stauungen eine Wirkung auf den durch Kalorien herbeigeführten Nystagmus erzeugen

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ERGEBNISSE

Afferente Neurone aus den Maculaorganen zeigen auch bei intakter efferenter Innervation eine spontane Aktivität von außerordentlich hoher Regelmäßigkeit. Wird die Lage des Tieres im Raum geändert, so ändern sich im allgemeinen auch die Entladungsraten. Der neue Wert wird nach einer geringgradigen und wenige Sekunden andauernden phasischen Aktivierung bzw. Hemmung erreicht und konstant eingehalten. Darüber hinaus können *auch bei längerer Beobachtungszeit keine weiteren Adaptationserscheinungen* gefunden werden. Die Veränderung der Entladungsrate hängt von der Kipprichtung ab. Für jedes Neuron läßt sich eine — und nur eine — Richtung finden, in der das Neuron maximal aktiviert wird. Bei Kippung in die entgegengesetzte Richtung wird das Neuron gehemmt. Diese Richtung maximaler Aktivierung ist für jedes Neuron verschieden.

Die Abbildung 1 zeigt Originalregistrierungen. Bei dem hier gezeigten Neuron war eine maximale Aktivierung bei Kippung nach vorn zu beobachten. In der Mitte ist die Aktivität des Neurons in Normalposition des Tieres dargestellt. Darüber ist die Entladungsfolge bei Hebung des Kopfes um 30° darunter bei Senkung um 30° zu sehen. Die außerordentlich große Regelmäßigkeit der Entladung ist auf den ersten Blick zu erkennen.

Die Aktivität eines Neurons bei verschiedenen Kippstellungen läßt sich in einem Zylinderkoordinatensystem darstellen. Eine solche Darstellung der Aktivität desselben Neurons zeigt Abbildung 2. Wie man sieht, ist jede

Z52-1-13



KIPPUNG
30 KOPF ↑

Z52-1-1



NORMALPOSITION

Z52-1-18



KIPPUNG
30 KOPF ↓

05 sec

Abb. 1 Originalregistrierung einer primären Maculaafferenz bei Normalposition (*Mitte*), Hebung des Kopfes um 30 (*oben*) und Senkung des Kopfes um 30 (*unten*)

physiologische Befunde von Schmidt (1963), von Gleisner & Henriksson (1963) sowie von Sala (1965) vor.

Über die funktionelle Bedeutung der efferenten Innervation am Maculaorgan liegen noch keine deutbaren Befunde vor. Rupert *et al.* (1962) haben primäre afferente Vestibularisfasern der Katze bei intakter efferenter Innervation untersucht. Sie beschreiben Neurone mit regelmäßigen Entladungen, deren *Entladungsrate sich mit der Kippstellung* ändert. Komplette Arbeitsdiagramme fehlen jedoch. Weitere Untersuchungen an primären Vestibularisfasern der Katze wurden von Wing (1963) veröffentlicht. Er hat bei seinen Versuchen aber das Vorderhirn abgesaugt und die Katzen in Arbeitsdiagramme fehlen jedoch. Weitere Untersuchungen an primären rente Innervation intakt geblieben ist. Er kam zu dem Ergebnis, daß das Otolithenorgan rudimentär und ohne nennenswerte Funktion sei. Cramer (1962) hat offenbar von Neuronen höherer Ordnung abgeleitet.

Es war das Ziel der hier beschriebenen Versuche, die Beobachtungen von Rupert *et al.* (1962) zu ergänzen, das Ergebnis von Wing (1963) zu überprüfen und die von Löwenstein & Roberts (1950) gefundenen Funktionsdiagramme der Maculaafferenzen bei erhaltener efferenter Innervation zu untersuchen.

METHODE

Die Untersuchungen wurden an Meerschweinchen durchgeführt. Zwei orientierende Versuche wurden an Katzen vorgenommen. Die Tiere wurden zunächst mit einem kurz wirksamen Thiobarbiturat (Trapanal 30 mg/kg i.p.) narkotisiert. *Es erfolgte eine Tracheotomie und die Einlage einer Beatmungskanüle.* Die Tiere wurden sodann mit Flaxedil relaxiert und künstlich beatmet. Sämtliche Operationsstellen und alle Druckstellen, die der Kopfhalter verursachte, wurden sorgfältig mit Xylocain lokal anästhesiert. Außer der Anfangsdosis wurde kein Narkotikum nachgegeben. Die Barbituratwirkung war zum Zeitpunkt des Beginns der Registrierungen abgeklungen. So konnte eine Depression der narkoseempfindlichen efferenten Aktivität (Gleisner & Henriksson 1963) vermieden werden. Nach Eröffnung der Schädelkalotte über dem Kleinhirn wurden die Tiere auf einem *kardanischen Tisch gelagert.* Die *Körpertemperatur* der Tiere wurde durch eine Heizung konstant gehalten.

Der kardanische Tisch, der um alle drei Achsen des Raumes zu kippen war, erlaubte es, das Tier in jede beliebige Position zu bringen. Nach dem stereotaktischen Atlas von Rössner (1965) wurden die auf das Kerngebiet zulaufenden Fasern des Nervus vestibularis mit metallgefüllten Mikropipetten aufgesucht. Die Aktivität dieser Neurone wurde bei verschiedenen Kippstellungen untersucht. Nach jeder Änderung der Kippstellung wurde 60 Sekunden abgewartet, um irgendwelche dynamischen Komponenten mit Sicherheit auszuschließen. Teilweise wurde nach dem Versuch die Lage der Mikroelektrode durch Koagulation markiert. Die Aktionspotentiale wurden mit einem Digitalcomputer (LINC 8) ausgewertet.

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Z52-1 13



Z52-1-1



Z52-1-18



0,5 sec

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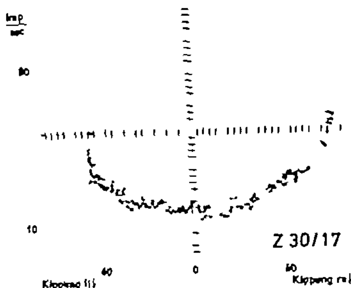


Abb. 3 Entladungsrat ines Neuron mit minimaler Aktivität bei Normalposition. A to-
matische A   rl g mit einem   Herrn Klauske geba ten lektronischen Zähler

handelte Ihre Aktivität war durch Berührung der homolateralen Kopfpar tie zu steigern Die Elektrodenspitze lag somit in diesen Fällen nicht im richtigen Gebiet

Die von uns beobachtete Änderung der Entladungsrate bei Kippung des Tieres ist gut korrelierbar mit den Potentialänderungen des Bestandspotentiales im Sinneszellager der Macula utriculi bei künstlicher Abscherung des Ot lithen (Trincker 1959 196)

DISKUSSION

In Analogie zu den Bogengängen kann angenommen werden daß es immer dann zu iner Aktivierung im primären afferenten Neuron kommt, w nn da Zilienbündel der zugehörigen Rezeptorzellen in Richtung des Kinoziliums abgeschert wird Nun ist die Orientierung der Zellen im Sinnesz ell erband der Maculae nicht inheitlich Nach polarisationsoptischen Untersuchungen v n Spoendlin (1964 1965) können praktisch alle Richtungen des dreidimensionalen Raumes al Orientierungsrichtung auftreten, wenn man di Macula utriculi und die Macula sacculi als funktionelle Einheit zusammenf ßt Die Abb. 4 zeigt die Orientierung der Kinozilien auf den Maculae nacl Befunden von Spoendlin (1965) Wenn man annimmt, daß eine afferente Faser jeweil einige benachbarte und damit gleich orientierte Sinneszellen versorgt, dann la sen sich unsere neurophy iologischen Befunde zwanglos den anat mischen Befunden on Spoendlin (1965) zuordnen Nach ihnen sind in der Tat sehr viele erschiedene Richtungen maximaler Akti-

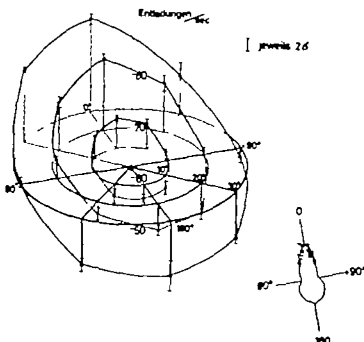


Abb. 2 Arbeitsdiagramm eines primären afferenten Neurons aus dem Maculaorgan eines Meerschweinchens bei Kippung in verschiedene Richtungen

Kippstellung mit einer bestimmten Entladungsrate korreliert, doch ist die Korrelation nicht umkehrbar eindeutig bei der Kippung nach halblinks bzw halbrechts ergeben sich ungefähr gleiche Entladungsraten Die einzelne afferente Faser besitzt also nur eine eindimensionale Richtcharakteristik, wie sie auch von Görner (1963) am Seitenlinienorgan von Xenopus laevis beschrieben wurde.

Neben den Neuronen, deren Aktivität bei Kippung nach vorn maximal zunahm konnten, wie schon erwähnt auch andere Typen gefunden werden, die bei Kippung nach seitlich oder hinten maximal aktiviert wurden. Schließlich wurde auch ein Neuron gefunden das in der Normalposition des Tieres eine minimale Aktivität zeigte zwei andere Neurone zeigten in Normalposition des Tieres eine maximale Aktivität. Wurde das Tier aus der Normalposition gekippt, so nahm unabhängig von der Kipprichtung, einmal die Aktivität zu im anderen Fall nahm sie ab Die Abbildung 3 zeigt ein Neuron mit minimaler Entladungsrate in Normalposition Beim Kippen sowohl nach rechts als auch nach links nahm die Aktivität zu Das galt auch für Kippung nach vorn und hinten

Spontan stumme, also in Normalposition inaktive Neurone wurden nicht beobachtet

Doppelentladungen, wie sie von Rupert *et al* (1962) beschrieben wurden konnten von uns nicht beobachtet werden Wir fanden zwar Neurone die Doppelentladungen mit sehr kurzem Intervall aufwiesen, sie zeigten auch eine Abhängigkeit von der Tierlage Bei genauerer Analyse zeigte sich jedoch immer daß es sich um Einheiten aus dem Kerngebiet des Trigeminus

wenstein & Roberts (1950) am isolierten Rochenlabyrinth und unseren Befunden am Meerschweinchen Sofern die Ergebnisse vom Rochen mit denen am Meerschweinchen überhaupt vergleichbar sind ist bei statischen Reizen auf den Otolithenapparat kein Einfluß der Efferenz auf die Entladung im afferenten Neuron festzustellen Es stellt sich also um so mehr die Frage nach der funktionellen Bedeutung der efferenten Innervation.

Innerhalb gewisser Grenzen ist das Vestibularorgan ohne Zweifel in der Lage Information über die Stellung im dreidimensionalen Raum zu liefern. Ein dreidimensionaler Raum wird durch drei linear unabhängige Vektoren aufgespannt. Wenn die primären Afferenzen — wie aus unseren Untersuchungen hervorgeht — ein eindimensionale Richtcharakteristik besitzen, dann mü sen mindestens drei verschiedene Typen von afferenten Fasern bzw Rezeptoren existieren, die sich in ihrem Arbeitsbereich, nicht aber in ihren prinzipiellen Eigenschaften unterscheiden, und die die drei linear unabhangigen Vektoren repräsentieren Sowohl nach unseren Untersuchungen als auch nach den Befunden von Spoendlin (1965) ist das der Fall wenn man Utriculus und Sacculus als funktionelle Einheit auffaßt Das System i t sogar mathematisch überbestimmt. Im übrigen dürfte der Utriculus die größere biologische Bedeutung besitzen

Der Ansicht von Wing (1963) daß die Maculaorgane bei der Katze rudimentar und ohne nennenswerte funktionelle Bedeutung seien, vermögen wir un nicht anzuschließen

W d ken Herrn I I Doz. Dr O J Grüsser fü die Anregung zu den *Untersuch* gen sowie für die t tkräftige U terstützung der Arbeit. Fra A. Thiele *danken* w für Il sorgf ltige tech ische Assi tenz, Herrn H. Dan enberg für den Ba des kardanischen Tisches.

SUMMARY

Guin pigs were placed n k rd nic t bl which permitted the positioning of the an mals in ll possible spatial *directions.* Action potentials of i gle primary f bers were recorded E en with intact *efferent innervation* th fibers how function l h racteristics imila t those described by Löwenst in an l Roberts for the isolated labyrinth of th ray Th *primary fibers,* which are supposed to supply several neighbou i g nd he ce similarly oriented receptors, po sess o i-d me sion l d rection l characteri tics. *Thus at least* three receptor types are requ red for the unequivocal locali n *f position in three-dimensional* space V rious t pes of rec ptors were ctually *found* which apparently correspond to the morphologicall orie ted cells i the sensory pithelium of the maculae A the operation l h racteri tics of the primary fibers re the same with and without ntact ff rent in ervation, the *influence* f the fferent fibers i therefore not et pparen t

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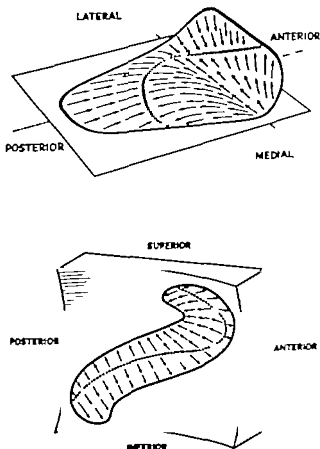


Abb. 4 Orientierung der Kinozilien auf der Macula utriculi (*oben*) und der Macula sacculi (*unten*) nach Spoendlin (1965) Der Pfeil weist in Richtung des Kinoziliums.

vierung zu erwarten Auch die von uns gefundenen Neuronentypen die bei Normalposition des Tieres eine minimale bzw maximale Aktivität aufweisen sind nach den Befunden von Spoendlin (1965) zu fordern Es sind Afferenzen von den Sinneszellen der Macula sacculi die so orientiert sind daß ihr Kinozilium bei Normalposition des Tieres ungefähr nach oben bzw nach unten zeigt

Selbstverstandlich konnte es sich bei den von uns gefundenen Neuronen auch um die von Löwenstein & Roberts (1950) beschriebenen „out of position und into level Neurone handeln Diese zweifellos besonders geartete Neuronenklasse besteht jedoch möglicherweise aus Afferenzen von der Lagena (MacNaughton & McNally 1946) die beim Sauger fehlt Wir sind daher der Ansicht, daß die von uns gefundenen bei Normalposition minimal bzw maximal aktiven Neurone mit solchen Rezeptorzellen verschaltet sind auf die der Otolith — wahrscheinlich der Sacculusotolith — in Normalposition einen minimalen bzw maximalen Reiz ausübt die sich aber sonst ebenso verhalten wie die übrigen Neurone Diese Hypothese konnte jedoch nicht endgültig überprüft werden da im Versuch der Kontakt zu diesen Neuronen jedesmal bereits bei einer Kippstellung von ± 60 verloren ging Abgesehen von dieser noch nicht endgültig geklärten Frage besteht jedoch eine auffallende Übereinstimmung zwischen den Befunden von Lo-

wenstein & Roberts (1950) am isolierten Rochenlabyrinth und unseren Befunden am Meerschweinchen Sofern die Ergebnisse vom Rochen mit denen am Meerschweinchen überhaupt vergleichbar sind ist bei statischen Reizen auf den Otolithenapparat kein Einfluß der Efferenz auf die Entladung im afferenten Neuron festzustellen. Es stellt sich also um so mehr die Frage nach der funktionellen Bedeutung der efferenten Innervation

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W d nken Herrn Priv Doz. Dr O J Grü ser für die Anregu g *zu den Untersuchungen* sowie fu die tatkräftige Unterstüt ung der Arbeit. *Frau A. Thiele* d nken wi für di sorgf ltige technische A sistenz, Herrn H Dannenberg für den Bau des kardanischen Tisches.

SUMMARY

Guinea pigs were plac d on a kardanic tabl which permitted the positi ning f the animals in all *possible spatial directions. Action* potential of single prim ry fibers were *recorded* Even with intact efferent in ervation the fibers h w functional characteristics simila to those described by Löwenstein and Robert for th solated labyri th *of the ray The primary* fibers, which are supposed to supply several n ighbouring and hen e similarly ori nted recept rs, possess ni-dimensional directional haracteristics. *Thus* t least three recept types are required for the unequivocal location *f position i* three-dimensional space Various types f receptors were ctually *found* which apparently correspond to the morphologically orient d cells in the *sensory epithelium* f th maculae As the operation l characteristics f the *primary fibers* are the same with and without intact ff rent innervation, th influen e *of the efferent fibers* i ther fore not yet pparent.

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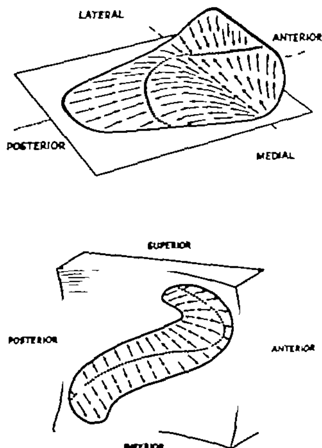


Abb. 4 Orientierung d Kin zili n auf der M cula utriculi (ben) und d r Macula sacculi (unt n) na h Spoendlin (1965) Der Pfeil w ist in Ri htung des Kin iliums.

vierung zu erwarten Auch die von uns gefundenen Neuronentypen, die bei Normalposition des Tieres eine minimale bzw maximale Aktivitat aufweisen sind nach den Befunden von Spoendlin (1965) zu fordern Es sind Afferenzen von den Sinneszellen der Macula sacculi die so orientiert sind, daß ihr Kinozilium bei Normalposition des Tieres ungefähr nach oben bzw nach unten zeigt

Selbstverständlich konnte es sich bei den von uns gefundenen Neuronen auch um die von Lowenstein & Roberts (1950) beschriebenen out of position und into level Neurone handeln Diese zweifellos besonders gear tete Neuronenklasse besteht jedoch möglicherweise aus Afferenzen von der Lagena (MacNaughton & McNally 1946) die beim Säuger fehlt Wir sind daher der Ansicht, daß die von uns gefundenen bei Normalposition minimal bzw maximal aktiven Neurone mit solchen Rezeptorzellen verschaltet sind auf die der Otolith — wahrscheinlich der Sacculusotolith — in Normalposition einen minimalen bzw maximalen Reiz ausübt, die sich aber sonst ebenso verhalten wie die übrigen Neurone Diese Hypothese konnte jedoch nicht endgültig überprüft werden da im Versuch der Kontakt zu diesen Neuronen jedesmal bereits bei einer Kippstellung von + 60 verloren ging Abgesehen von dieser noch nicht endgültig geklarten Frage besteht jedoch eine auffallende Übereinstimmung zwischen den Befunden von Lö-

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HEARING LOSS AND CALORIC RESPONSE IN MENIERE'S DISEASE

A Comparative Study

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In 343 patients with Menière s disease the caloric response was compared with the hearing loss as measured by pure ton audiometry and a distinct correlatio was found. With increasing tone threshold the caloric reaction from the ffected ear tended to become weaker and the number of patients with a reduced caloric reacti n increased One hundred d thirty-eight patients underwent speech udiometry and the maximal discrimination was calculat d. No correl tion was found between discriminati n loss and reduction of th caloric reaction The tracing width of the Békésy audiogram on stimulation with continuous tone wa compared with the loric rea tion in 53 patients. \ correlation wa found. The relationship between the separatio between the thresholds f r continuous and interrupted t ne in Békésy audiometry and the caloric reaction was studied in 40 p tients. A disti ct correla tion was found. Th greater th separation between the tw Békésy recordings, the greater often was the reduction f the caloric response

Characteristic of Menière s disease i a disturbance of both the cochlear and the vestibular function. It is not yet established, however to what ex tent lesions in the cochlea and labyrinth are from a functional aspect, mutually connected. In two previous studies the caloric reaction (Stahle & Bergman, 1967) and the pure tone audiogram (Enander & Stahle, 1967) have been treated separately It was evident from these investigations that the majority of the patients had an abnormal caloric response but that this response was reduced in only about half of them All patients had a sensory neural hearing loss, and the mean value for the tone thresh ld in 334 pa tients was 52 dB Among the pure tone audiograms the most common type of curve was the "flat loss type" (60%) The hearing loss seemed to occur mainly during the first year of the disease after which there was only a small increase with time No special configuration of the pure tone audiogram, pathognomic for early or late stages of the disease was found.

Recruitment of loudness, first described by Fowler (1936) is today a well known phenomenon in Menière s disease and other disorders of hearing f a cochlear origin (Dix *et al.*, 1948) In 1947 Békésy showed that in hearing loss due to cochlear lesions the tracing width of the Békésy audio-

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Hz, always in the direction from low to high frequency The interruption rate for the interrupted tracing was 2.5 i.p.s.

For speech discrimination tests the Swedish PB word lists were used. The level of presentation was 30 dB above the speech reception threshold When the discrimination could not be determined at that level because of discomfort the discrimination tests were presented at three to four different levels and an articulation curve was plotted

Caloric testing

The caloric tests were performed with water at 30 C and 44 C, and in cases where no reaction was obtained with these stimuli, iced water was used. The patients lay in the horizontal supine position with the head raised 30 (Stahle 1956 Stahle & Sahl 1964) The reactions were recorded by means of electronystagmography (Aschan *et al* 1956)

The cal ric responses were a sessed exclusively on the basis of the eye speed in the slow nystagmus phase which is a considerably more sensitive indicator of labyrinthine function than the duration of the response (Henriksson, 1956 Stahle 1956 1958, 1968) The mean eye speed during a 10-second period at the peak of the reaction was assessed and denoted maximum intensity" As a measure of the caloric response the percentage difference between the maximum intensities from the right and left ear was calculated, according to principles given by Jongkees & Philipszoon (1964) The normal limit for this difference wa placed at 20% (= 2.5 times the standard deviation)

MATERIAL

A total of 343 patients were investigated, and all of these exhibited the typical triad of symptoms. The average duration of the disease was 8 years. The series included 227 patient who had had such severe vertigo that after the examination they underwent an operation (ultrasonic irradiation) All patients were tested with pure tone audiometry and electronyst gmograp hy by uniform methods.

For studies of Békésy audiograms, those patients were selected f r whom recordings had been made with a Grason Stadler E-800 audiometer Since this apparatu had been available for only a short time this series was limited. The tracing width was calculated for 53 patients, and the separation between the recordings of continuous and interrupted tone was studied in 40 patients.

Speech discrimination, finally had been measured in 138 of the patients

RESULTS

Pure Tone Hearing Loss and Caloric Response

The hearing loss in 343 patients was correlated with the difference between the al ri reactions from the two labyrinths. The hearing loss was calculated from the mean value of the ir conduction at frequencies of 500 1000, and 2000 Hz. The mean hearing loss for the entire series was

gram was narrowed in the frequency region for which the patient showed recruitment. This observation has since been supported by Lundborg (1952) who investigated 26 patients with Menière's disease and found that all of them exhibited narrowing of the tracing width. Békésy considered that narrowing of this width constituted indirect proof of recruitment. This relationship has been questioned, however, by Hirsh *et al.* (1954), Landes (1958) and Jerger (1962).

With the method introduced by Jerger (1960) in Békésy audiometry in cochlear damage a separation is often found between the recordings of continuous and interrupted tone within the high frequency range. Harbert & Young (1962) have pointed out that early cases of Menière's disease often show normal amplitude and superposition of continuous and interrupted tracings, and late cases show reduced amplitude and separation between interrupted and continuous tracings.

The speech discrimination loss in Menière's disease has been the subject of several studies, and reports in the literature show a discrepancy between the results of different investigators. While some authors (Passe 1953, Lidén 1954) have found that poor discrimination is a typical feature, others have found that the discrimination is only moderately or slightly impaired (Opheim & Flottorp 1957, Jerger 1960). An articulation curve of a hump-back type has been considered to be typical of Menière's disease (Cawthorne & Harvey 1953). On comparing the speech discrimination loss and hearing loss for pure tones, Cawthorne & Harvey (1953) found no constant relationship and Goodman (1965) found that they could fluctuate independently of one another.

Studies with light microscopy have shown changes especially in the cochlea and vestibule and to a smaller extent in the semicircular canals, and large individual differences can occur (Kristensen, 1961, Lindsay 1960, 1966, Schuknecht 1963, Altmann & Kornfeld 1965). Our own studies of the function of the inner ear have shown that permanent cochlear functional loss is more common than vestibular functional loss (Stahle & Bergman 1967). Whether the reason for this is that in certain cases the disease affects the cochlea to a higher degree or that the semicircular canals are more resistent, is not known.

The aim of the investigation reported below was to study retrospectively in a large series of patients, the relationship between loss of cochlear function and loss of function of the semicircular canals.

METHODS

Hearing tests

The pure tone audiograms were recorded with an Amplivox (Model 61) audiometer and narrow band masking was used. Békésy audiometry was carried out with a Grason Stadler E-800 audiometer. The attenuation rate was 2.5 dB per second. Sweep-frequency tracings for both interrupted and continuous tone stimuli were obtained for the frequency range 125–8000

Hz, always in the direction from low to high frequency The interruption rate for the interrupted tracing was 2.5 i.p.s.

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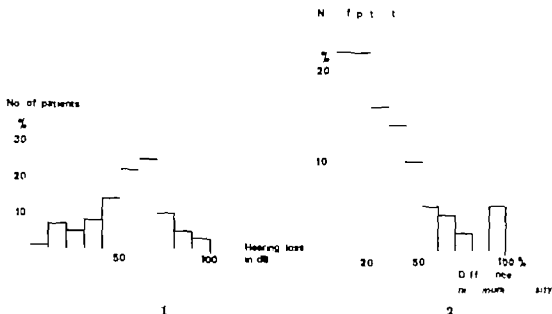


FIG. 1 Percentage distribution of 343 patients with Menière s disease accordi g to hear ing loss.

FIG 2. The results of the cal ric test in 343 patients with M nière s disease xpressed as th percentage difference in the maximum intensities recorded from th right and left sides Th normal limit fo thi differe ce is set at 20% Th percent g distribution f patients is given on the ordin te

53 4 dB The distribution of patients according to degree of hearing loss can be seen in Fig 1 The difference in maximum intensity for the 343 patients was, on the average 29% the distribution of patients according to degree of reduction of the caloric response is shown in Fig 2. The relation between caloric response and hearing loss can be seen in Fig 3, which is based on a classification of the patients into 5 groups according to degree of hearing loss 0–19 dB 28 patients 20–39 dB 47 patients 40–59 dB 123 patients 60–79 dB 119 patients and 80–100 dB 26 patients. The mean values for the maximum intensity differences in these 5 groups were 16 5 18 1 29.5 33 7 and 40.5 and these values are plotted in the figure There was a distinct but weak statistical correlation between the magnitude of the hearing loss recorded and the reduction of the caloric response The coefficient of correlation was 0 31 which is significant at a significance level of 5%

In order to elucidate these questions further the 343 patients were divided into 4 groups according to their degree of hearing loss, and the caloric reaction in the different groups was then studied (Fig 4 *a* *b* *c* and *d*) Out of 30 patients with a hearing loss not exceeding 20 dB (Fig 4 *a*) the *caloric reaction was normal in 25 and reduced in 5 On the other hand,* among 135 patients with a hearing loss of 61 dB and over (Fig 4 *d*) the caloric reaction was normal in 44 and reduced in 91 Thus with an increasing degree of hearing loss the number of patients with a reduced caloric response increased

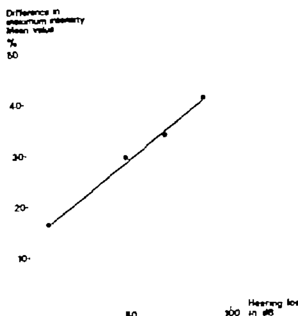


Fig. 3. The relation between hearing loss and caloric response (difference in maximum intensity see text i Fig 2) i 343 patients with Ménière' disease

Maximum Discrimination Score and Caloric Response

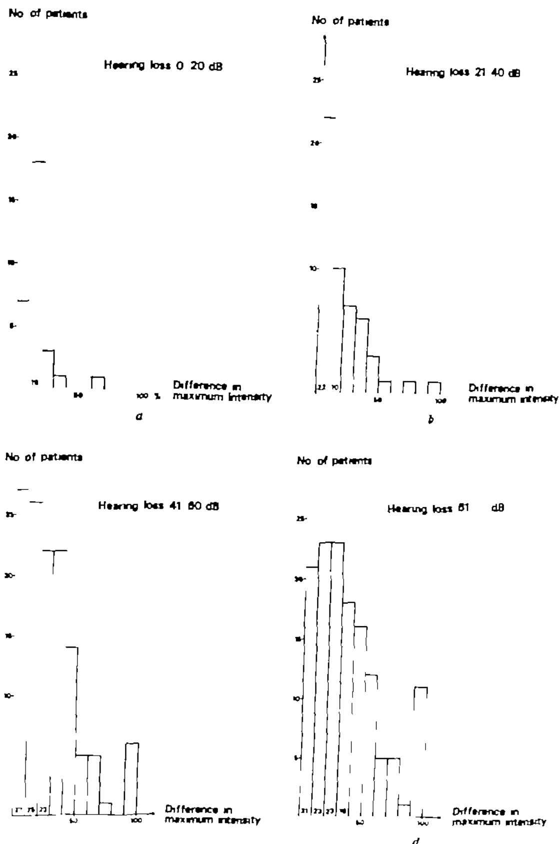
The maximum discrimination score in 138 patients was, on the average 53%. The distribution of patients with different degrees of discrimination loss is shown in Fig 5. As can be seen in Fig. 6, no constant relationship between the maximum discrimination score and the caloric response was found. Neither was any correlation shown on statistical analysis.

Békésy Audiometry and the Caloric Response

(a) The tracing width of the Békésy audiogram and the caloric response

According to Lundborg (1952) the decrease in the tracing width of the continuous tone found in patients with Ménière's disease occurs as a rule in the frequency range of 1000 Hz and upwards. We therefore calculated the mean width of the tracing in the frequency range 1000–8000 Hz for each patient. A comparison based on the results from 53 patients is shown in Fig. 7. It is evident from this figure that in patients with a narrow tracing width, both normal and greatly reduced caloric reactions can be seen. No correlation was found on statistical analysis.

P lva (1956) suggested that recruitment should be considered to be present when the tracing width of the continuous tone is less than 5 dB. Of the 53 patient studied, 28 showed a mean width of the tracing of less than 5 dB within the frequency range 1000–8000 Hz, while 25 patients had



F a 4 a–d Th lori response (difference in maxim m int nsity see t t t F g 2) in 4 group of pati t with different degrees f hearing loss. With n incre i g degree of h ri g lo the n mber of patients with a reduced l ri respo se t d t increase The num ral in th colum represent th umber f pati ts.

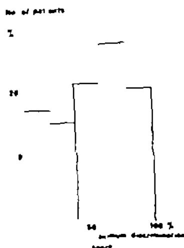
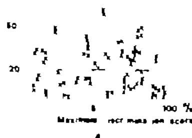


Fig. 5. The percentag distribution of 138 patients according t ma im m discrimination core

a mean width of 5 dB or more The maximum intensity difference for the former group was 29% and for the latter group 27% this minor difference was not statistically significant.

(b) The separation between interrupted and continuous tracings in the Békésy audiogram and the caloric response

The study of the separation was limited to the frequency range 500–8000 Hz, since Owens (1964) pointed out that in cochlear lesions the threshold



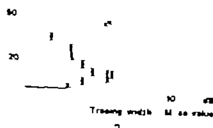


Fig. 6 Correl tion bet een the maxim m discrimination score and the caloric response (difference in maxim m intensity) in 138 Menière patients. N indication of any constant relationship between these tw factors is shown. The dotted line indicates the normal limit in the caloric response

Fig. 7 Comparison betwee the tracing width of the Békésy diogram nd the caloric response (ordinat) in 53 patients. No correlation is show

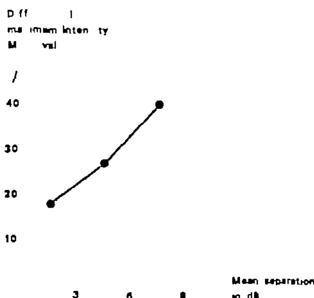


Fig 8 Comparison between the mean separation of interrupted and continuous tracings in Békésy audiogram (*abscissa*) and the caloric responce (*ordinate*) in 40 patients. A distinct correlation is shown

shift of the steady tone Békésy tracings was less reliable in frequencies below 500 Hz. The separation was measured from the bottom of the spikes. The mean separation within the frequency range 500–8000 Hz was calculated for each patient and correlated with the maximum intensity difference in a total of 40 patients As can be seen in Fig 8 there is a clear correlation Fig 8 was based on the following mean values Group 0–3 dB, 8 patients with a difference in maximum intensity of 17 8% 3–6 dB 16 patients with a difference of 27 0% 6–9 dB, 16 patients with a difference of 39.8% Statistical analysis showed a correlation coefficient of 0 27 which means significance at a significance level of 10% It is concluded that the greater the separation the greater the tendency to a reduction of the caloric response

COMMENTS

Histopathological findings reported in the literature seem to indicate that the semicircular canals are affected by the lesion to a different degree from the cochlea and vestibule A common finding in studies of recent years is that the disease affects predominantly the membranous walls, while on light microscopy no marked changes have been noted in the neural structures Distension bulging, and ruptures of the membranous walls have been shown in the cochlea and vestibule in particular but only to a small extent in the semicircular canals. The functional disturbances are considered to be due both to biochemical alterations in the labyrinthine fluids and to a direct mechanical effect on the sensory end-organs in connection with ruptures (Schuknecht 1963 Altmann & Kornfeld, 1965) Lindsay (1960) has gone one step further and assumed that this is a question of different mechanisms

of attack in the cochlea and labyrinth, where the auditory disturbances are due to alterations in the chemical composition of the labyrinthine fluids, while vestibular manifestations are an expression of a direct mechanical effect of the collapsed membranous labyrinth on the vestibular end-organs

In the light of reports of certain differences in the histopathological picture and different theories as an explanation for functional changes in the cochlea and labyrinth, we considered it of interest to present a clinical study of the relationship between auditory and vestibular function. Our study was based, however on the results of only a few selected function tests, and the comparison must therefore be regarded as incomplete

Th results of our investigation indicate that there is a relationship between hearing loss, recorded by pure tone audiometry and the caloric response The greater the increase in the tone threshold, the greater the tendency to reduction of the caloric response and the greater the number of patients with a reduced response. When, on the other hand, the tone threshold was raised to only a minor extent, the caloric reaction was often normal. It should be emphasized that in approximately 40% of the patients the caloric reaction was normal in spite of a hearing loss in all cases. This lack of agreement can probably be explained primarily by the inadequacy of the technique for examination of the semicircular canals. As a secondary explanation, however the possibility should be considered that in many cases the disease attacks the cochlea to a greater degree than the semicircular canals. This latter theory may have some support in observations at light microscopy

A weak but distinct relationship was shown, further between the threshold shift of continuous tone in sweep-frequency Békésy tracings, and the caloric response. Usually the greater the separation between the continuous and the interrupted Békésy tracings, the greater was the reduction of the caloric response On the other hand, no correlation was found either between the caloric response and the discrimination loss or between the caloric response and the tracing width of the continuous tone in Békésy audiometry

The reason for the finding that certain hearing tests show a correlation to the caloric response, while others show no such correlation must be that the different hearing tests reflect different aspects of the hearing function According to Goodman (1965) the hearing level for tones and the discrimination loss for speech in Menière s disease can fluctuate independently of one another Similar observations have been made previously by Passe (1953) Goodman considers that these findings "lend support to the possibility that different and probably independent sets of conditions underlie hearing-los for tones and discrimination loss for speech" Huizing & Reyntjes (1952) demonstrated a close relationship between discrimination loss and recruitment. The pathological loudness function, i.e. recruitment, leads to di tortion with consequent discrimination loss. Recruitment is considered generally to be a manifestation of hair cell damage The reduced amplitude

of the Békésy audiogram has been considered to be an indirect recruitment test, but this has been questioned during recent years. The threshold shift of the continuous Békésy tracings is an expression of adaptation (Jerger 1960). Studies of cochlear microphonics have shown, however, that adaptation is not a phenomenon associated with the hair cells.

In view of the above reports, the reduced width of the tracing in Békésy audiometry and also the discrimination loss in speech audiometry could partially reflect hair cell damage. Our investigation showed that there was no significant relationship between the results of those hearing tests in which the changes could largely reflect hair cell damage, and the caloric response.

On the other hand, we did find a correlation between the caloric response and those hearing tests in which the change in Menière's disease reflected a direct hair cell lesion to only a minor extent, but where the lesion lay at another, perhaps more central level in the cochlea (pure tone audiogram and separation). With our as yet limited knowledge of the neurophysiology of the inner ear and of the basic cause of the recruitment phenomenon, however, these assumptions can only be considered hypothetical.

ZUSAMMENFASSUNG

Bei 343 Patienten mit Menièrescher Krankheit wurde die kalorische Reaktion mit dem tonaudiometrisch gemessenen Hörverlust verglichen und ein eindeutiger Zusammenhang konnte festgestellt werden. Je höher die Tonschwelle lag, desto schwächer war meist auch die kalorische Reaktion des kranken Ohrs. Um diesen Zusammenhang deutlicher in Erscheinung zu bringen, wurden die Patienten dem Hörverlust entsprechend in vier Gruppen eingeteilt und die kalorische Reaktion wurde in den verschiedenen Gruppen festgelegt. Unter 30 Patienten mit einer Hörverschlechterung von höchstens 20 dB konnte nur in 5 Fällen eine Herabsetzung der kalorischen Reaktion festgestellt werden. Unter 136 Patienten mit einer Hörverschlechterung von über 60 dB wurde Herabsetzung der kalorischen Reaktion in 91 Fällen nachgewiesen. Mit zunehmendem Hörverlust nahm also die Anzahl von Fällen mit herabgesetzter kalorischer Reaktion zu.

138 Fälle wurden der Sprachaudiometrie unterzogen und die maximale Diskrimination wurde berechnet. Ein Zusammenhang zwischen Diskriminationsverlust und Herabsetzung der kalorischen Reaktion konnte nicht nachgewiesen werden. Bei 53 Patienten wurde die Höhe der Spitzen in den Békésy Diagrammen mit der kalorischen Reaktion verglichen. Ein Zusammenhang konnte nicht nachgewiesen werden. Geringere Höhe der Spitzen konnte sowohl bei Patienten mit normaler als auch bei Patienten mit fortgeschrittener Abnahme der kalorischen Reaktion auftreten. Der Zusammenhang zwischen den Abständen der Schwelle für kontinuierlichen und gepulsten Ton bei Békésy Audiometrie einerseits und der kalorischen Reaktion andererseits wurde bei 40 Patienten untersucht. Ein deutlicher Zusammenhang konnte nachgewiesen werden. Je grösser der Abstand zwischen den zwei Aufnahmen bei Békésy Audiometrie war, desto grösser war meist auch die Herabsetzung der kalorischen Reaktion.

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HEARING REHABILITATION BY MEANS OF TRANSDERMAL ELECTROTHERAPY IN HUMAN HEARING LOSS OF SENSORINEURAL ORIGIN

Preliminary Report

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An amplitude modulated radio frequency energy method (the TD system) is described which was applied to the problem of the rehabilitation of sensorineural hearing loss. The TD system was tested on a group of 40 patients with moderate to severe sensorineural hearing loss using a double blind experimental design. The treated group showed a statistically significant improvement in PB word discrimination scores when compared to the control group. Evidence is presented to show that speech discrimination hearing is possible by means of TD electrostimulation in a totally deaf patient.

Over 200 patients with moderate to severe sensorineural hearing loss have been studied during the course of the development of techniques for hearing rehabilitation by means of electrostimulation. The majority of patients have experienced various degrees of improvement in hearing with electrostimulation superior to that found with other conventional means (Puharich & Lawrence, 1964). No undesirable side effects have been found to date as a result of electrostimulation. Clinical experience with electrotherapy in patients with sensorineural pathology by Puharich & Lawrence (1964), Manfredi & Bombelli (1963) and Rollenburd (1964) indicates this to be a promising new technique with potentialities for hearing rehabilitation.

The signal generator is a power amplifier of an amplitude modulated double side band low frequency carrier signal. The output of the generator is coupled to the skin of the patient through an LC series resonant network, and each line terminates on the skin with a plate electrode. Electrodes can be of bare metal, or metal covered with a dielectric material, or a combination of each type.

The electrodes of the transdermal (TD) system are placed across the head of the patient. The carrier signal is brought to peak resonance and is modulated with an audio frequency band that sweeps from 100 Hz to 10 KHz and back again with a four-minute cycling rate. Each discrete audio frequency is cycled every one second from 0–100 per cent modulation

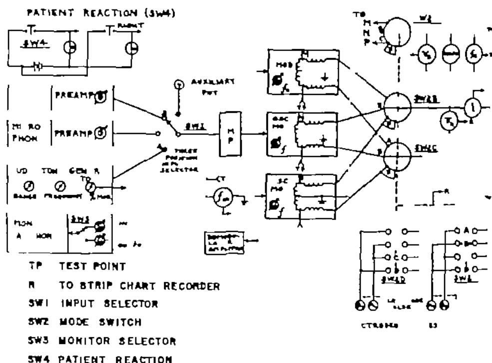


FIG. 1 Clinical transdermal hearing system diagram

and back to 0 per cent with a triangular AM envelope This energy is applied across the head for a period of about one hour each day for variable periods of time See Fig 1 for circuit block diagram

EXPERIMENTAL DESIGN AND METHOD

The null hypothesis was "Efficacy of TD treatment *is not* due to electrotherapeutic effects." Alternate hypothesis "Efficacy of TD treatment is not due to experimental error on the part of the investigators" and/or Efficacy of TD treatment is not due to experimental error on the part of the patient This hypothesis was tested by exposing patients to a double-blind TD therapeutic regimen in which pairs of patients would be on placebo or TD treatment for one week

Patients were selected and evaluated by three independent Board Certified Otolaryngologists, and referred to the experimental TD program at Intelectron Three audiologists, all Certified for Clinical Competence carried out the audiometric measurements for the three otologists Each otologist audiologist pair worked independently of each other The Intelectron staff audiologist, Diana Meyer Certified for Clinical Competence replicated audiometric tests on all of the patients referred by each otologist Thus, there was obtained an independent set of data by one audiologist on all the patients measured by the three other audiologists The authors managed the TD treatment program The distribution of sensorineural hearing loss (as SRT) before th   xperiment (control) in the 28 patient sample is shown in Fig 2

Two TD tre tment apparatuses were used as follows

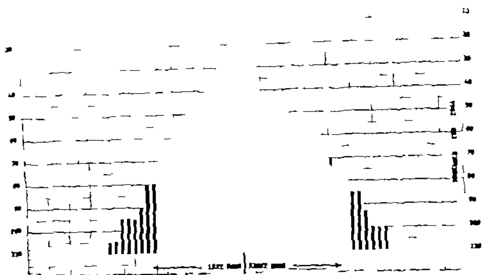


FIG. 2. SRT data (control) taken before manipulation experiment. Histogram showing distribution of SRT levels in 28 patients. The histogram shows normal distribution curve for bilateral moderate to severe sensorineural hearing loss. Tail end data below abscissa indicates no response (n.r.) of patient at 110 dB

1 Two patients were placed on each clinical TD unit at one time One patient received TD therapy and the other one received an acoustic signal through the headset as a placebo treatment Both patients wore identical headsets.

2. Setting of the crypto unit in the clinical TD unit was made by a disinterested party and this code was sealed for the first week of treatment.

During the week prior to start of treatment, each patient had two complete audiometry tests. The treatment cycle as outlined in Table 1 was given over the period of four and one half days. One half day was allowed for the patient to visit his otologist for audiometry tests.

At the end of the first week, the seal on the TD crypto unit was broken and it was determined which patient had been on treatment. During the second week, the patient who had been on placebo was TD treated, and the treated patient was kept on TD treatment for a second week. The following week was used to conduct comprehensive audiometric tests for comparison with the initial tests.

No patient was informed before, during, or after the experiment that this was a double blind experiment. In the treatment program outlined in Table 1 it is to be noted that the control of electrode modes and head position of the electrodes was automatically carried out by a tape program See Fig 1 circuit. TD treatment and placebo treatment was identical with respect to audio bandwidth, audio frequency shifts, and audio volume rise and fall

TABLE 1 *Clinical TD treatment program for only one day*

Step	(min) Time	Electrodes	Head position S.M Right	Head position S.M Left	Head position, tragus Right	Head position, tragus Left	Cyclical AF mod. (Hz)	Series inductance (mH)
1	0–10	Case I A	x ←	→ x			2000–20	70
2	10–20	Rest						
3	20–30	Case I A	x ←	→ x			8000–2000	2
4	30–40	Rest						
5	40–50	*Case III*	*Bare* ←		→ *Coated*		*200–8000*	30
6	50–60	Rest						
7	60–70	Case III		Bare ←		→ Coated	200–8000	30
8	70–80	Rest						
9	80–90	Case III	Bare			Coated	200–2000	30
10	90–100	Rest						
11	100–110	Case III	Bare			→ Coated	2000–10,000	30
12	110–120	Rest						
13	120–130	Case III		Bare ←	→ Coated		200–2000	30
14	130–140	Rest						
15	140–150	Case III		Bare ←	→ Coated		2000–10 000	30
16	150–160	Rest						
17	160–170	Case II			x ←	→ x	20–200	30
18	170–180	Rest						
19	*180–190*	*Case II*			x ←	→ x	*200–10 000*	30
20	190–200	Rest						

since these were taped in advance and, therefore constant for every patient No speech education or therapy was included in the treatment program. Table 2 shows the total time of active treatment each patient had on the TD machine during this program

Audiometric Tests

A Pure tones

Air Conduction 250 Hz, 500 Hz, 1 KHz, 2 KHz, 4 KHz, and 8 KHz.

Bone Conduction 250 Hz, 500 Hz, 1 KHz, 2 KHz, and 4 KHz.

The three outside audiologists used manual techniques testing each ear separately *employing white noise masking where applicable* descending modified Hughes & Westlake technique The Intelectron audiologist used an automatic audiometer which uses a modified bracketing technique both ascending and descending each ear tested separately Critical band masking applied when necessary

B Speech audiometry

1 Speech reception threshold—descending technique (in 5 dB steps) testing each ear separately threshold recorded at 50 per cent correct at minimal dB level

TABLE 2 *Patient data.*

Female			Male		
Code no.	Age	TD Rx time, hrs	Code no.	Age	TD Rx time hrs
21	28	6.5	24	77	6.5
12	65	6.5	27	61	6.5
17	54	6.5	31	82	6.5
19	19	6.5	30	74	6.5
20	55	13	23	33	13
29	16	13	10	54	13
25	18	13	7	37	13
6	57	13	13	84	13
15	38	13	28	55	13
11	67	13	26	58	13
9	59	13	18	57	13
5	21	26	1	67	27
2	72	26	4	84	26
14	40	26	8	75	9
No. of females: 14			No. of males: 14		

2. Speech detection threshold—descending technique (in 5 dB steps) testing each ear separately threshold recorded at minimal level at which speech was discernable though not intelligible

3. Speech tolerance threshold—ascending technique (in 5 dB steps) testing each ear separately to level at which speech becomes uncomfortable

4 Speech discrimination scores

(*a*) Discrimination tested at 40 dB above speech reception threshold level, each ear separately Per cent score recorded based on number per 25 words correct. Masking employed when necessary in contralateral ear

(*b*) Discrimination at PB maximum determined by increasing or decreasing input in 10 dB steps to level at which patient achieves optimum discrimination pe cent out of 25 words. Each ear tested separately masking when applicable

(*c*) Discrimination at 45 dB dial reading when possible 25 words presented to each ear and per cent correct recorded

We were interested in those systematic differences that might exist between Intelectron audiometric procedures and those of the participating audiologists in the general sense For this reason, a set of averages were computed fo each pre-treatment test administered by our staff audiologist, and a corresponding set across all three participating audiologists. We focused on the before treatment period since we were interested solely in audiometric, and not treatment, effects. The null hypothesis here is that regardless f the test being admini tered, variations in the measuring pro-

TABLE 3 *(1 and 3) Audiologists matched t tests—PB Pct*

A = Intelectron data, *A* = other's data.

	Control week 0	*Control*, week 0–1	*Control*, weeks 1–2
Mean	−4.993	0	4.8 5
s.D	14 6"5	11 "30	14 1"6
N	15	16	16
Difference	−4 093	0	4 8"5
s.E.	3 789	2.933	4 282
D F	14	15	15
t−test	−1 302	0	1 139
p =	0.14	1 000	0.2731
	A −*A*	*A* −*A*	*A* −*A*

There is i significant difference between th data of Int lectron audiologist on th pati nt sample, and the data of th other three audiologists on the same patient sampl

cedures are randomly distributed, resulting in no significant differences between either set of average scores.

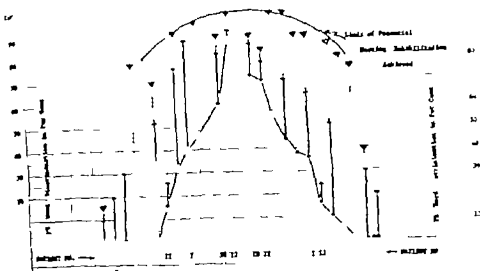
The *t* statistic was used to test for differences between uncorrelated means under the *t* distribution We accepted the alternate hypothesis, that a differ ence between staff and participant average scores as large as measured was due to real effects, when the probability of our being wrong in our accept ance was five in a hundred This level of significance was our criterion for all statistical tests carried out in this analysis

We did not assume homogeneity of variance between our two sets of data (i e., that the distribution of individual scores within an average was similar in shape across a set of means) and used the Welch (1947) approximation for heterogeneous variances in all tests of significance between audiologists.

In the ideal experimental design, a complete set of data is collected from each randomly selected subject Because of the pilot nature of this study it was not possible to meet this criterion One reason for missing data was in the limits of the audiometer With a power range of 0–110 dB, we were limited to using data from those subjects who responded within these limits. Where subjects did not respond, they were scored no response scores.

The final sample size listed with each *t* test computation accounts for those subjects with complete sets of data for that particular test The com puter program which produced our statistics (the data text system)[1] automatically eliminates a subject from the test if any part of his needed responses are incomplete This does not preclude his contribution to tests of remaining scores in the series.

Compute used *IBM 7094 II* *Computer program used* *Th* *dat t xt st m.* (1) Comp t ti n f *t* t betwee ind pe dent groups, (2) comp t ti n f *t* test betwee matched lsers t



I ro 3. Symbolcode PB discrimination in with TD acoustic stimulation (post-treatment); T PB discrimination in with acoustic stimulation (post-treatment); PB discrimination in with acoustic stimulation (pre-treatment).

In planning the experiment we took advantage of the reduction in error variation inherent in employing repeated measures. In this design, the required number of groups are created on the basis of equivalency and random selection. Then after each treatment session, the same group is repeatedly measured for the effect in question. In order to determine if changes have occurred, the t test for correlated means is used. It differs from the uncorrelated version described earlier in that error variation due to non-treatment effect are reduced, thereby increasing the size of the t value. Non-treatment effects would be those related to subject idiosyncracies such as test fatigue, nervousness, or other responses which exist across all test situations.

RESULTS

When we compared audiometric data done by the Intelectron audiologist vs. outside audiologists on the same patient sample with matched t-tests, we found that the two sets of data are homogenous for the 1 variables of interest. However when we look for deviation from week 0 as a result of experimental manipulation during week 0–1, weeks 1–2, and weeks 0–2, we find a consistent deviation in only one of the 15 variables, namely PB scores given in percentages. Therefore we look at the audiologists PB's matched t-tests in Table 3 for the three time periods in question, week 0, week 0–1 and weeks 1–2. We find that the two sets of data are homogenous, and, therefore we can analyze either set of data with confidence.

When we look at the outside audiologists data in Table 4 we see that

TABLE 4

1 Treated group

	PB % (other's data)	
	TD treated, week 0–1	TD treated, weeks 1–2
Mean	−7 077	0.545
S.D	9 987	10.511
N	13	11
Difference	−7 077	0.545
S.E.	2.770	3 169
D F	12	10
t−test	−2.555	0.172
p=	0 012	0.5 (approx.)

2 Placebo group

	PB % (other's data)	
	Placebo, week 0–1	*TD treated, weeks 1–2*
Mean	−2.800	−2.429
S D	6.877	8.121
N	10	14
Difference	−2.800	−2.429
S.E.	2.175	2.171
D F	9	13
t−test	−1.288	−1 119
p=	0.115	0.161

the treated group for week 0–1 shows significance at the 1 per cent level, and the placebo group for week 0–1 is not significant (at the 11 per cent level) However this result is based on a small n In order to further test this result we found an equivalent small n from the Intelectron audiologist s data by using either left ears, or right ears as data source Table 5 shows such right ear data for week 0–1 It is to be observed that the outside audiologists data and the Intelectron audiologist s data are equivalent. Thus, both sets of data show a real treatment effect at work with TD therapy and none in the placebo group

The power of this argument is amplified in Table 6 where a larger n is obtained by using only the Intelectron audiologist s data Using Welch s solution (1947) *it is found that the critical ratio of difference between* the treated group (15 33 PB%) and the placebo group (4 81 PB%) is significant at less then the 1 per cent level It is concluded that this statistical analysis reveals a real treatment effect for only one of the 15 audiometric variables in the course of a two-week period of experimental manipulation However it turns out that with respect to the end goal of any hearing rehabilitation therapy the one variable of utmost importance is that of speech

TABLE 5

Right ear only — PB Pct. (Intelectron data)

Treated group

	Week 0–1	Weeks 1 2
Mean	−12.800	−8.000
S.D.	12.621	8.000
N	10	8
Difference	−12.800	−8.000
S.E.	3.991	2.828
D F	9	7
t−test	−3.207	−2.828
p =	0.011	0.025

Right ear only — PB Pct. (Intelectron data)

Placebo group

	Placebo week, week 0–1	Treatment week, weeks 1 2
Mean	−4.727	−7.000
S.D.	9.089	9.440
N	11	10
Difference	−4.727	−7.000
S.E.	2.741	2.985
D.F	10	9
t−test	−1.725	−2.345
p	0.115	0.044

discrimination enhancement. This was the variable that showed a significant enhancement with TD therapy as measured by the PB test.

It was considered desirable to prolong the course of TD therapy in order to see if any of the other variables, in addition to PB% showed any significant deviation. This question is examined in the next section on a new sample of 12 patients. Since placebo therapy had not shown any significant deviations in the 28 patient sample, the double-blind design was not used in the next study

LONG TERM TD THERAPY

Summary of findings

(*a*) Twelve cases ranging in age from 18 to 83 years with various degrees of sensorineural hearing loss ranging from − 40 dB to − 110 dB average for pure tones were studied to evaluate potential efficacy and hazard of TD electrostimulation.

(*b*) TD electrostimulation was given t these patients on the basis of one hour per day TD therapy for 12 to 72 treatments over periods ranging from one day to six months.

(*c*) Pure tone audiograms were done before TD therapy was initiated, at suitable intervals during therapy and after therapy had ceased. Not a

TABLE 6

1 Treated group

	PB % (Intelectron data)	
	TD treated, week 0–1	TD treated weeks 1–2
Mean	−15.333	−6.429
S.D	13 958	1_.507
N	18	14
Difference	−15.333	−6.429
S.E.	2.290	3.343
D F	17	13
t−test	−4.661	−1 923
p =	0 0001	0 01

2 Placebo group

	PB % (Intelectron data)	
	Placebo week 0–1	TD treated, weeks 1–2
Mean	−4 818	−6.600
S.D	.823	10.918
N	22	20
Difference	−4 818	−6.600
S.E.	1 608	2.441
D F	21	19
t−test	−2 889	−_ 03
p =	0.005	0.001

C.R of difference for 0–1 w k — t for group 1 vs group 2 (Welch Sol)

t_{obs} = −3 00 p = < 01 [$t_{.01}$ (30) = 1 70 t = 34 1].

single patient showed any loss at any frequency with respect to residual acoustic hearing function as a result of prolonged electrostimulation No undesirable side effects of electrostimulation were observed or reported

(d) A criterion of ±0 dB was established as a variation for significant improvement or significant loss for pure tone thresholds. The audiogram spectrum was divided into three categories, i e low frequency range (125–750 Hz) middle frequency range (1–5 kHz) high frequency range (6–8 kHz) This criterion was applied in each of the three categories to assess improvement or loss in pure tone thresholds following TD therapy Table 7 shows the results in tabular form It was concluded that (1) no patient suffered a significant loss in threshold level in any of the three categories, (2) that the greatest lowering of threshold occurred in the low frequency category and (3) that the middle frequency range is the least affected by TD electrostimulation

(e) For speech audiometry a criterion of ±10 per cent was established as the 1 per cent level of significance It is found that eight of the ears fall

TABLE 7 *Pure tone changes*

+ 6 dB, or better improvement; 0 6 dB, or less, change; − = 6 dB, or more loss. AD = Right Ear AS = left Ea

Case	LI (125 Hz–750 Hz)		MF (1 Hz–5 kHz)		HF (6 kHz–8 kHz)	
	AD	AS	AD	AS	AD	AS
1	+	+	+	0	+	+
2	+	+	+	0	+	+
3	+	0	0	+	0	0
4	0	0	0	0	0	0
5	+	+	+	+	+	0
6	+	0	0	0	0	0
7	+	+	0	0	0	+
8	+	+	0	0	+	+
9	+	0	+	0	+	0
10		+	0	0	0	0
11	0	0	0	0	0	+
12	0	0	0	0	0	0
Totals for twelve patients						
+	9	6	4	2	5	5
0	3	6	8	10	7	7
−	None	None	None	None	None	None
Totals for twenty-four ears						
	15		6		10	
0	9		18		14	
−	None		None		None	

below the 1 per cent level and 16 of the ears exceed the 1 per cent level of significance. It was concluded that 66 per cent of the ears show significant improvement for acoustic speech discrimination

(*f*) When we assess acoustic speech discrimination scores before therapy s. TD electrostimulation reinforced by an acoustic source after therapy fi e of the ears fall below the 1 per cent level of significance, and 19 of the ears exceed the 1 per cent level. This data is summarized in Fig. 3

(*g*) It was found that PB speech audiometry tests could be done at significantly lower le els >6 dB during the post therapy period as compared to the pre-therapy period.

CASE REPORT

R. J is a 26-year-old white male Scarlet fever mumps, and meningitis during th age from two to three years left him totally deaf R. J s eyesight was seriously affected. H was left with minimal residual vision until the age f 20 when he became totally blind

It is w ll documented during all the years since R. J s meningitis that

TABLE 8 *Protocol of defective hearing cases currently under clinical TD therary*

PB tests are always given in blocks of 50 taped words from standard lists, xcept where noted by an asterisk. An aste isk refers to blocks of 10 PB words which the patient learns, and the test is given by randomizing the word order

				PB s.D before R		PB speech discrimination post R, Acoustic		Transdermal + acoustic		
Case no	Diagnosis	Age	No. of R	AD %	AS, %	AD %	AS, %	AD %	AS,	Binaural,
1 (S I R.)	AD SN loss. AS deaf (Menière s)	70	72	36	0	64	42	90	40	96
2 (R B)	Congenital SN loss (moderate)	18	12	54	46	96	88	100	96	100
3 (R Mc.)	Infectious SN loss (severe)	38	20	0	0	42	38	76	78	82
4 (P A)	SN loss sec ondary to otosclerosis	27	30	44	32	70	76	100	92	100
5 (S. L.)	AD presby AS deaf (30 yrs dur)	72	24	10	0	50	10	86	16	83
6 (J v H)	SN loss sec. Neomycin. Ototoxicity	48	26	6	0	70	52	80	0	80
7 (G N)	SN loss. Presbyacus. (mod rate)	83	36	38	40	82	88	90	88	92
8 (F S.)	SN loss. Bilat ral (total)	58	25	PB = 0 0 Spondi =	 0	 30 18	 30* 12	 40 20	 40* 20	 40* 25
9 (M P)	SN loss. Bilateral (t tal)	52	49	0 Spondi =	0*	20 0	20 0	20 16	20* 12	20* 16
10 (D T)	SN loss. Presby us. (mild)	77	49	72	60	86	82	90	90	80
11 (J W)	Unsymmetric. b lateral SN loss	56	22	70	10	78	26	84	30	86
12 (W F)	U ymm tr b lat al (SN loss)	49	14	16	88	24	92	20	100	100

A la to-l yng 6

he has been totally anacoustic for pure tones in the frequency range from 125 Hz to 8 kHz at 110 dB pressure levels. His sensitivity to skin vibration was also tested and it was noted that he could sense vibrations up to 1 / KHz.

The use of two bare electrodes was found to be the best technique to deliver the sensation of sound to R J when they were placed bilaterally behind the ear ½ to ¾ inch below the mastoid prominence The technique used 2 mH chokes and a carrier ranging from 17 kHz to 23 kHz. The current across the head was 2 mA at 2 volts with a total power of 4 mW or less than 1 mW/cm²

An initial test was made of the frequency range of TD response before transdermal therapy It was found that his response was from approximately 90 Hz to 2 6 KHz. From this time on, successive visits found his range of TD response increased from 70 Hz to 8 kHz. This range was reached in no more than six or seven office visits each lasting no longer than one and one half hours.

With the same bare electrode technique we began to speak words into a microphone and at the same time R. J was made aware of each word by placing his finger across the speaker's lip chin, and throat. Initially we started with a group of five or six monosyllable words such as *cat dog* etc R. J could learn such a group of words within ten minutes, and when given to him at random, he could score with 70 per cent to 80 per cent accuracy

This lead to more complicated groups such as *fun gun run sun* etc. He could repeat these with equal accuracy No attempt was made to teach R J the English language Words led to sentence acquisition which he could learn as readily as single words and repeat with similar accuracy (≈ 80 per cent)

Since R. J has been with us for only five months, it is difficult to say at this time what the extent of his language learning capability can be via transdermal hearing. Much work remains to be done At the end of the five-m nth period, R. J is still anacoustic to 110 dB pure tone levels from the acoustic udi meter

CONCLUSIONS

A novel electrotherapeutic technique the Transdermal system (TD) was dev loped. It wa applied to the problem of hearing rehabilitation in hear ing loss f sensorineural rigin in a group of 40 patients.

Twenty-eight patients with sensorineural hearing loss were subjected to a one week double blind course of TD electrotherapy Patients receiving placebo therapy showed an average five per cent increment in PB word discrimination scores, and this is ascribed to test, retest learning (Group 2) P tients receiving active TD electrotherapy howed an averag 17 per cent increment in PB word discrimination scores (Group 1) The critical rati f difference between Group 1 and Group 2 is significant at $p = < 0.01$

Twelve other patient with sensorineural hearing los were then subjected

TABLE 8 *Protocol of defective hearing cases currently under clinical TD therary*

PB tests are always given in blocks of 50 taped words from standard lists, except here noted by an asterisk. An asterisk refers to blocks of 10 PB words which the patient learns, and the test is given by randomizing the word order

Case no	Diagnosis	Age	No of *R*	PB s D before *R* AD %		PB speech discrimination post *R* Acoustic AD %	AS, %	Transdermal + acoustic AD	AS, %	Binaural
				AD %	AS, %	AD %	AS, %	AD	AS, %	Binaural
1 (S. I R)	AD SN loss, AS deaf (Menière s)	70	72	36	0	64	42	90	40	96
2 (R. B)	Congenital SN loss (moderate)	18	12	54	46	96	88	100	96	100
3 (R Mc.)	Infectious SN loss (severe)	38	20	0	0	42	38	76	78	82
4 (P A)	SN loss secondary to otosclerosis	27	30	44	32	70	6	100	92	100
5 (S. L.)	AD presby AS deaf (30 yrs dur)	72	24	10	0	50	10	86	16	88
6 (J v H)	SN loss sec. Neomycin. Ototoxicity	48	26	0	0	70	52	80	70	80
7 (G N)	SN loss. Presbyacus. (moderate)	83	36	38	40	82	88	90	88	93
8 (F S.)	SN loss. Bilateral (total)	58	25	PB = 0						
				0	0	30	30	40	40	40*
				Spondi =		18	12	20	20	28
9 (M P)	SN loss. Bilateral (total)	52	49	0*	0	20	20	20	20	20*
				Spondi =		0	0	16	12	18
10 (D T)	SN loss. Presbyacus. (mild)	77	49	72	60	86	82	90	90	90
11 (J W)	Unsymmetric bilateral SN loss	56	22	70	16	8	26	84	30	86
12 (W F)	Unsymmetric bilateral (SN loss)	49	14	16	88	24	92	20	100	100

ZUSAMMENFASSUNG

El e amplitudemodulierte Radiofrequenz Energie-Methode (das sogenannte TDS-System) ist geschildert die beim Problem der Rehabilitation (bzw Wieder instandsetzu g) des sensori euralen Hörverlustes angewandt wurde Das TDS-System wurde an einer Gruppe von 40 Patienten ausprobiert die in eine Probegruppe und eine Kontrollgruppe ingeteilt waren Diese P tienten hatten mäs igen bis starken Hörverlust der sensory Nerven. Die behandelte Gruppe zeigte ine statistisch bedeutende Verbesserung gegenüber der Kontrollgruppe in PB-Wort unterscheidungs-Ergebnissen E wird bewiesen, d ss sprachunterschidendes Hören bei einem vollkommen t uben Patienten möglich ist mit Anwendung der TD-Elektrostimulierung.

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to a prolonged course of TD electrotherapy They showed an average 2/ per *cent increment in PB word discrimination scores.*

Ten animals were studied in the following three areas TD electrostimulation of hearing in two totally deaf dogs showed evidence for hearing neuropathological studies of the brains of two dogs subjected to chronic (320 hours) TD electrostimulation revealed no gross or microscopic abnormalities and cardiovascular physiological studies of acute (eight hours) TD electrostimulation in six dogs showed no abnormal effects It is concluded that TD electrostimulation is safe for humans within the power and time exposure applied

A case report is given on the results of TD electrotherapy and TD hearing electrostimulation with a totally deaf patient Evidence is presented to show that speech discrimination hearing is possible in a totally deaf patient by means of TD electrostimulation

There are two main mechanisms to consider The first is to try to explain sensorineural rehabilitation resulting from TD electrotherapy The second is to explain speech discrimination in the totally deaf human by means of TD electrostimulation

With respect to the first, human and animal studies have shown that the application of amplitude modulated AC energy (the TD signal) to tissues creates a DC barrier potential of 0.5 volt The induced barrier potential is negative in sign inside of cells, and positive on the outside The exception to this rule is that red blood cells, leukocytes and platelets, accumulate a negative charge on the outside surface This effect prolongs blood clotting time *in vitro* by a factor of three compared to controls that were not charged with the TD signal Microscopic observation shows that TD charged red blood cells are repelled from each other by negative charge repulsive forces Rosen *et al* (1962 1964 1965) have stressed the importance of circulatory efficiency in the cochlear system with respect to good hearing and to hearing loss In personal communications with Dr Rosen, a plan was formulated to evaluate the effects of TD electrotherapy on cochlear circulatory factors This may be one of the more important effects of clinical TD therapy which leads to improved speech discrimination hearing

With respect to speech discrimination by means of TD electrostimulation in the deaf the problem is very complex. It has been established that the induced DC barrier potential is modulated by the audio envelope of the amplitude modulated carrier signal How this effect can substitute for the cochlear transduction and encoding processes is not yet clear Various models for such a cochlear function analog are now being explored by computer simulation One of the more promising models being investigated is the artificial presso-receptor analog of Teorell (1966)

such examples as the electromyographic studies described by Faaborg-Andersen (1965) on humans and the cortical study by Hast & Milojevic (1966) on the primate most of our knowledge of the physiological mechanisms of the intrinsic muscles of the larynx has been derived from research on the domestic dog and cat (Fessard & Vallancien, 1957 Floyd *et al* 1957 Dunker & Schlosshauer 1958 Isshiki, 1959 Mårtensson, 1963, 1964 Mårtensson & Skoglund, 1964 Kirchner & Wyke 1965 Hast, 1961 1964 1966 *a*, 1966 *b* 1967 *a* 196 *b*) This fact does not necessarily lessen the value of past experimental findings. But this researcher believed that it would be most fruitful to expand our explorations into the realm of man's closest homologue. We are fortunate in having as our base the excellent comparative anatomical studies of Sir Victor Negus (1929) and the reader is referred to this text as a source for a detailed description of most animal larynges.

The purposes of this study were (1) To measure the mechanical properties of the thyroarytenoid and cricothyroid muscles in three primates, the Hynan gibbon (*hylobates hainanus*) rhesus Macaque (*macaca mulatta*) and squirrel monkey (*saimiri sciureus*) and (2) to compare findings from primate research to previously published data derived from experiments on dogs and cats.

MATERIAL AND METHOD

Because of the great expense and difficulty in acquisition, only two gibbons (an anthropoid ape) were used in this study the male weighed 7 kg and the female 4 kg Experiments were also performed on 5 rhesus squirrel monkeys of both sexes their average body weight was 3 0 kg (range of 3-4.3 kg) and 670 g (range of 400-850 g) respectively All animals were anesthetized intraperitoneally with pentobarbital sodium (20 25 mg/kg) As a consequence of the size of the laryngeal organ in the rhesus and squirrel monkey (Fig 1) all operative procedures on these animals were performed with the aid of a Zeiss operation microscope and special microsurgical instruments.

Animals were placed in a supine position, tracheostomy was performed to maintain a free airway and the larynx exposed from the hyoid bone to the second or third tracheal ring. The recurrent laryngeal and extrinsic branch f the uperior laryngeal nerve was freed unilaterally by use of a ery fine dissecting scalpel and dissecting needle

A ventromedian incision was then made through the larynx from the hyoid to the first tracheal ring. This was followed by two mediolateral incisions f the thyroid cartilage one superior and one inferior to the vocal fold the origin of the thyroarytenoid muscle was finally freed by section ing the thyroid cartilage perpendicular to and between the mediolateral incisions. A length of silk was tied into the cricoid attachment of the cricothyroid muscle and another into the thyroid attachment of the thyroaryte

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THE PRIMATE LARYNX

A Comparative Physiological Study of Intrinsic Muscles

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A study was made of the mechanical properties of the thyroarytenoid and cricothyroid muscles in three primates Squirrel monkey rhesus Macaque and Hynan gibbon The thyroarytenoid and cricothyroid muscles were stimulated electrically through their respective motor nerve supply Isometric contractions of muscles were recoded by a force transducer and high-speed inkwriter Measurements were made of the contraction time half relaxation time tetanus, and tetanus twitch tension ratio of each muscle It was found that the thyroarytenoid possessed the properties of a "very fast" muscle (14 msec) and the cricothyroid of the gibbon and rhesus those of a muscle of average" speed (38 msec) With the exception of the cricothyroid muscle in the squirrel monkey (19 msec) there were no real differences among the three primates studied Comparative values of both laryngeal muscles were similar for the domestic dog and primate but not for the cat whose laryngeal muscles had been found to be physically slower The physiological similarity of the dog and the primate larynx substantiates the dogs value as an experimental analogue for studies in muscle physiology of the larynx

When one considers that the greater part of our knowledge of the science of physiology is derived from observations of laboratory experimentation on animals, it behooves the researcher to continually reinvestigate his principal source of material through a study of comparative anatomy and physiology Aside from the idealism of seeking a basic truth the medical scientist assumes a responsibility when pursuing his discipline the aquisition of knowledge for the betterment of human health Hence the scientist attempts to apply findings of basic research to man He must, however relate experimental data cautiously fully aware that differences between species may limit the application of comparative findings to human function

The abov discussion is of singular importance in the study of the neuromuscular mechanisms of the laryngeal organ With the exception of

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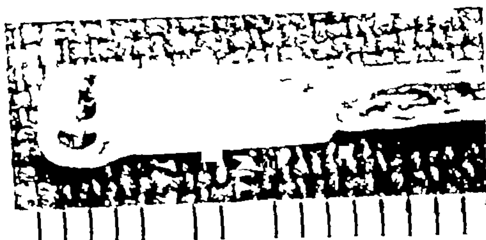


FIG. 2. The stimulating electrode used in this study Dimensions of the electrode-head 2.7 mm 3 mm.

traction was established, the amplitude of the electrical stimulus was increased to a supramaximal voltage of 1–2 V The nerves were stimulated with square wave pulses of a duration of 0 1 msec at a frequency of 1–150/sec (Grass S4G stimulator) Trains of stimuli were limited to 2 sec and an interval of 10 sec was maintained between successive tetani to allow for dissipation of post tetanic potentiation of muscle twitches.

RESULT

The results of all experiments are given in Table 1 Examples of mechanical properties of each muscle are shown in Figs. 3, 4 and 5

An examination of the data reveals that for gibbon, rhesus, and squirrel monkey the average contraction times for the thyroarytenoid muscle were similar One variant should be noted, however the very fast response of 8 msec by one of the squirrel monkeys. There was also no real difference between average values for the mechanical properties of the cricothyroid muscle in the gibbon and rhesus the notable exception was the faster contraction time of the squirrel monkey The mean values from this author's past research, published (Hast, 1966, 1967 a) and unpublished, on dogs and cats is presented in order that a comparison can be made between these two common laboratory animals and the three primates. An examination of Table 1 revealed that mechanical properties of the dog's laryngeal muscles were quite similar to that of the primates this is not true for the thyroarytenoid and cricothyroid muscles of the cat

Not shown in Table 1 was the force exerted by these muscles during an isometric contraction at *in situ* resting length. For the rhesus monkey the average tetanus twitch tension ratio (g wt.) was 51 4 g 7 1 g for the

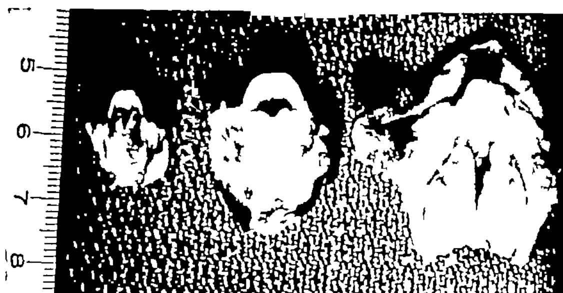


FIG. 1 A dorsal view of the three primat laryngec studied From left to right: Squirrel monkey rhesus, and gibbon

noid muscle To further isolate the thyroarytenoid muscle from the inter action of other intrinsic muscles, the arytenoid cartilage was sutured to the thyroid cartilage With the gibbon ties were made with 4–0 surgical suture because of the limitations of size in the case of the rhesus and squirrel monkey ties were made with 6–0 silk armed with a micro-point cutting needle (Ethicon)

The free end of each piece of silk was tied to a force displacement transducer (Grass FT 0 03 c) attached to a heavy stand The output signal of the transducer was amplified and recorded by a high speed inkwriter (Offner RS Dynograph) Isometric recordings were made of each muscle separately For all primates, measurements of the mechanical properties of con traction time, one half relaxation time tetanus, and tetanus twitch tension ratio were taken at the *in situ* resting length of each muscle Since the speed of muscle contraction has a high temperature coefficient ($Q_{10} = 1\ 53$) according to Gordon & Phillips (1953) temperature was maintained at 37 C ($\pm 0\ 2$) by the heat of an electric lamp and monitored by a thermistor probe connected to the circuit of a calibrated electrical thermometer The preparation was bathed by a physiological irrigating solution It should also be noted that the *in situ* resting length was maintained since the progressive lengthening of a muscle would increase the contraction time of the twitch (Buller *et al.*, 1960)

The laryngeal nerves were attached to a very small shielded bipolar platinum electrode and bathed in a pool of warm mineral oil The electrodes used in this study were designed by the author and constructed by a skilled craftsman at our institution (Fig 2) [1] After the threshold of muscle con

[1] The lectrod h wn I Fig 2 w mad b M J h A Dys n, Ph I I gy Pharm col gy Sh p, Medi l Laborat ies, Uni rsity f I wa, I w City I wa.

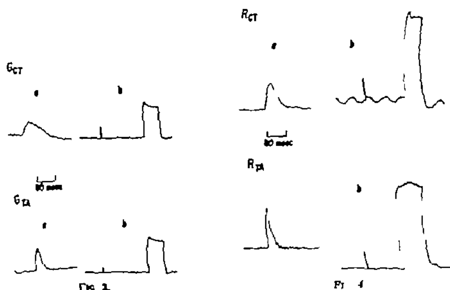


Fig. 3

Fig. 4

Fig. 3. Recordings of mechanical properties of two intrinsic laryngeal muscles of gibbo no. 1 G_{CT}, Cricothyroid muscl ; G thyroarytenoid muscle; ingle twitch response for calcul ting contracti nd half-relaxation times; b twitch nd tetanic responses from which the tet nus twitch tension ratio was computed.

Fig. 4 Recordi gs of mecha ical properties of tw intrinsic laryngeal muscles of rhesus no. 2: R_{CT}, Cricothyroid muscle; R_{A} thyroarytenoid m scle; a, singl twitch response for calculating contraction and half-relaxation times b twit h nd teta ic responses from which the teta us twitch tens on rati wa computed.

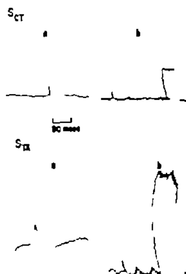


Fig. 5. Recordi gs of mecha ical properties of tw intrinsic l ryngeal muscles f squirrel monkey no. 4 S_{CT}, Cricothyroid m scle; S_{A} thyroarytenoid m scl ; a, single twitch response for calculating contraction and half-relaxation times; b twitch nd teta ic responses from which the tetanu t itch tension ratio computed.

TABLE 1 *Mechanical properties of the cricothyroid and thyroarytenoid muscles of the Hynan gibbon rhesus Macaque and squirrel monkey*

			Cricothyroid				Thyroarytenoid			
	Sex	Weight	Contraction time msec*	$^1/_2$-relaxation time msec	Tetanus (pulses/sec)	Tetanus Twitch tension ratio	CT	$^1/_2$-RT	Tet	T T
Hynan gibbon										
1	m	7 0 kg	40	36	40	3 0 1	16	16	100	6 2 1
2.	f	4 0 kg	48	36	45	3. 1	16	18	90	5 5 1
Average			39	30	43	3.4 1	16	17	95	5 9 1
Rhesus Macaque										
1	m	3 0 kg	28	20	60	5.4 1	12	16	90	.1 1
2.	m	3.4 kg	44	24	40	4.2 1	14	12	110	5 3 1
3	f	3.0 kg	38	24	45	4.5 1	14	16	110	6.1 1
4	f	4 1 kg	40	21	50	3 9 1	16	14	130	10.2 1
5.	m	1.3 kg	32	24	50	4.2 1	14	11	120	7 0 1
Average			36.4	23.2	40	4 4 1	14	14 4	112	1 1
Squirrel monkey										
1	m	850 g	22	14	65	4 1 1	16	14	90	4.4 1
2.	m	650 g	16	10	80	4 4 1	16	14	90	4.2 1
3.	f	800 g	24	14	70	4 2 1	12	16	120	5.8 1
4	f	400 g	16	12	90	4 6 1	8	10	150	9.6 1
5.	m	680 g	16	12	110	5.5 1	14	16	130	6.0 1
Average			18.8	13.6	83	4 6 1	13.2	14	116	6.0 1
Domestic dog (verage of 10)			39	32.3	45	4.2 1	14	18	114	6 2 1
Domestic cat (verage of 10)			52.8	42.1	37	3.7 1	22	20	73	5 0 1

* ±1 msec

thyroarytenoid muscle and 22 8 g 5 2 g for the cricothyorid muscle With the squirrel monkey the average tension was 3 4 g 610 mg for the thyroarytenoid muscle and 2 3 g 512 mg for the cricothyroid muscle These values for muscle tension serve to illustrate the several problems that can arise when dealing with such small masses of tissue, particularly in experiments with the squirrel monkey

DISCUSSION

With the exception of the cricothyroid muscle of the squirrel monkey average values for the mechanical properties of both muscles revealed no real differences among the primates studied Values for the average contraction times of 19 36 and 39 msec for the cricothyroid muscle of the

the two levels, each best suited to perform a particular function. By this means adaptations for a particular purpose can take place at one level without sacrificing anatomical requirements for a different but equally important function which can continue to take place at the second level. For instance had a single powerful sphincter band been retained, it would of necessity have been either too gross a structure to produce a pleasant variable voice or if it had developed as a single delicate band capable of producing graded tones over a long range such delicacy of structure would in all probability have rendered it ineffective as a powerful sphincter. It is, incidently interesting to note that the greater physiological importance of the sphincteric mechanism is hinted at in nature by the fact that in species in which the larynx is designed primarily for only one purpose, the anatomic structure of the organ is better adapted to the sphincter action than to the production of sound.

The very real differences observed in this study between the laryngeal muscles of the cat and those of man's closest homologues, the monkey and ape may open to question the value of the cat as an experimental analogue. On the other hand, the physiological similarity of the dog and the primate larynx substantiates the dog's value for research on the neuromuscular mechanisms of the larynx.

ZUSAMMENFASSUNG

Die mechanischen Eigenschaften der Thyreoarytenoideus- und Crieothyroideus-Muskeln wurden in drei Primaten untersucht: *Hylobates hainanus, Macaca mulatta, Saimiri sciurea*. Die genannten Muskeln wurden über ihr jeweiliges Bewegungsnervensystem elektrisch gereizt. Die isometrischen Kontraktionen der Muskeln wurden durch einen Kraft-Transducer und einen Hochfrequenz-Tintenschreiber aufgenommen. Gemessen wurden die Kontraktionszeit, die Halb-Relaxationszeit, die Tetanusfrequenz sowie das Tetanus-Zerspannungsverhältnis jedes Muskels. Der M. thyreoarytenoideus erwies sich als ein „sehr rascher“ Muskel (14 msec) und der M. cricothyroideus des Gibbon und Rhesusaffen als ein Muskel von „mittlerer“ Geschwindigkeit (38 msec). Mit Ausnahme des M. cricothyroideus des *Saimiri sciureus* (19 msec) verliefen die Untersuchungen mit den 3 Primaten ohne wesentliche Unterschiede. Vergleichswerte für die beiden Kehlkopfmuskeln bei Hunden und Primaten waren ähnlich, nicht aber bei Katzen, deren Muskeln sich bei früheren Versuchen als langsamer erwiesen haben. Die physiologische Ähnlichkeit der Kehlköpfe von Hund und Primat bestätigt den Wert des Hundes als experimentelles Analogon in Untersuchungen der Physiologie der Kehlkopfmuskeln.

squirrel monkey rhesus, and gibbon respectively do not agree with the published findings of 25 msec by Mårtensson & Skoglund (1964) A problem arises, though in trying to compare this work with that of Mårtensson & Skoglund (1964) for to use their own words, only certain muscles of the cat and dog were systematically studied [with] occasional readings made on monkey (The number and species of monkey was not given)

The squirrel monkey with its very high pitched voice not only possesses a "fast cricothyroid muscle but a vocal fold muscle capable of achieving an extremely high speed of contraction Judging from the 8 msec value of squirrel monkey no 4 In this particular animal the thyroarytenoid muscle was *comparable in speed of contraction to the extraocular medical rectus* of the cat (Cooper & Eccles, 1930)

One of the difficulties in attempting to correlate the data among the three animals is the limited number of the experiments performed on the gibbon This researcher believed however that since there has been little published data on the physiological properties of primate laryngeal muscle findings from experiments on this anthropoid ape, no matter how limited, would be of interest to physiologists and laryngologists

There is another observation which must be discussed and here again the reader is referred to Table 1 Values for the mechanical properties of the primates intrinsic laryngeal muscles were similar to those of the domestic dog but not the cat This investigator found the thyroarytenoid (Hast 1967 *a*) and cricothyroid (unpublished) muscles of the cat to be physiologically slower" than comparable muscles in the dog Mårtensson & Skoglund obtained reverse results Values for the contraction times of these laryngeal muscles in the dog (Hast 1966 *a* 1967) do agree however with the findings of Mårtensson & Skoglund (1964) One cannot account for the large and contrary differences found between this study and the previous work (cricothyroid 30–35 msec, thyroarytenoid 9–13 msec) for values of cat laryngeal muscles, especially since the techniques used by this researcher were quite similar to those of Mårtensson & Skoglund Again, it is difficult to compare studies, since those of the other authors reported values for only one mechanical property in contradistinction to the four measurements made on each muscle by this investigator

Comparative gross and microscopic anatomical studies, by this and other equally important function which can continue to take place at the physiological findings For example the laryngeal sphincter of the cat is represented by a single valve in contrast to the double sphincter (*false and true folds*) of the dog monkey ape, and man In their monograph Pressman & Kelemen (1955) offer some interesting speculations on the significance of this major structural and evolutionary difference

> It is well that in man such a division took place for it provides the advantage of permitting differences in anatomical construction at

the two levels, each best suited to perform a particular function. By this means adaptations for a particular purpose can take place at one level, without sacrificing anatomical requirements for a different but equally important function which can continue to take place at the second level. For instance had a single powerful sphincter band been retained, it would of necessity have been either too gross a structure to produce a pleasant variable voice or if it had developed as a single delicate band capable of producing graded tones over a long range, such delicacy of structure would in all probability have rendered it in effective as a powerful sphincter. It is, incidently interesting to note that the greater physiological importance of the sphincteric mechanism is hinted at in nature by the fact that in species in which the larynx is designed primarily for only one purpose the anatomic structure of the organ is better adapted to the sphincter action than to the production of sound.

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COMPTE RENDU

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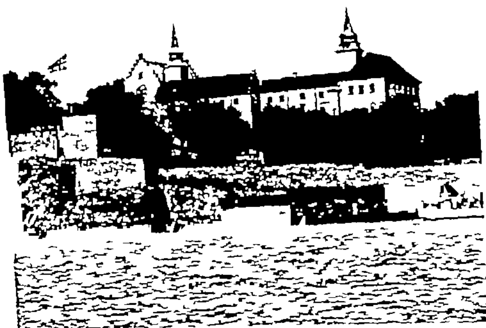
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UPPSALA 1969



INTRODUCTION

Hans Vogt

Oslo, Norway

Ladies and Gentlemen

The president of this distinguished society has asked me to say a few words at the opening of this congress. It was a pleasure to accept this invitation and to have the opportunity of bringing you the greetings of the University of Oslo, which I have the honour to represent.

Congresses have an important function in the scientific life in our time. The mass of information published every year is so enormous that no man can hope to cope with it singlehanded. The solution to this problem is very often personal contact with colleagues, co-workers, and friends who can share their knowledge so that each of them can pick up just that piece of information he needs. Congresses can serve the purpose of creating contact over and across national boundaries—on the condition however that they succeed in keeping the number of participants within reasonable limits. Con-

gresses with many thousands of participants—6000 mathematicians at the last international congress of mathematicians in Moscow—defeat their own purpose

You have, as far as I know in your society and at your annual meetings stressed quality more than quantity friendship and cooperation more than competition and rivalry That should in itself be a guarantee for the success of the work you will do in the days to come

On behalf of the University of Oslo I bring you best wishes for the congress, and declare the congress open.

Acta oto-laryngologica 67 97-100 (1969)

TITRATION OF HORMONE METABOLITES IN CASES OF NASOPHARYNGEAL ANGIOFIBROMA FIVE YEARS AFTER THE OPERATION

S. Karatay, S. Katircioglu and O. Erözden

Istanbul, Turkey

At the 1963 meeting seven nasopharyngeal angiofibromas were reported from the endocrinological point of view. Four cases out of seven were followed for five years. Recent investigations of their hormone metabolites revealed that in the tumor-free cases the hormone-metabolite titrations had improved. In cases with the tumor still present, findings were the same as at the beginning.

During the Collegium meeting in Athens in 1963 we reported an endocrinologic study of eight cases of nasopharyngeal angiofibroma concerning the *relationship between the sex hormones and the pathogenesis of the tumor* (Karatay *et al.*, 1963).

On that occasion we tried to demonstrate that there was always an androgen–estrogen imbalance in these cases. Upon the post-operative death of one of those cases we had the opportunity to study histologically the patient's endocrine glands and gonads (which were also described in the abovementioned paper).

The seven cases were kept under control for five years after the operation and they were given additional hormone preparations. Only four of them, however, came to their last control in 1968. As a complementary study to the first paper, this short contribution will deal with the follow up of the laboratory findings of these cases, obtained in the last controls.

As we stated before, we believe that nasopharyngeal angiofibroma is a male pathology. From 1955 up to 1968 we observed in our clinic 33 cases. The first female case was hospitalized only this year (1968) with a biopsy diagnosis of angiofibroma. After the operation material was once more sent for histological study and this time a *retinoblastoma* was diagnosed. Hormone metabolites of this case, which were studied before the operation, were also found normal.

LABORATORY FINDINGS

According to the findings of our follow-up cases, we can state that following surgical removal of the tumor and post-operative treatment with hormone preparations (Testoviron, 25 mg twice a week for a period of 3–4 months) the androgen–estrogen balance returned to normal after a time in the tumor-free cases.

TABLE 1

Case		1 Co mg	17 OH mg	Folliculin-gamma
1	8	2 10–4.88	1.37–.61	–8–26
2.	5	4 90–8.5	3.96–7.5	27–20
3	4	5.2 –9 7	3.43–8.5	35–15
4	2	5.13–0 80	2.8 –5.45	30–70
Normals		12–18	5–9	0– gamma pre puberty 5–15 gamma puberty 10–20 gamma adults

8, 5, 4 2 are the case numbers from the original paper (1963) The first column in each titrated hormon metabolites corresponds to the preoperative finding, whereas the second column shows the findings five years after the operati ns.

On the other hand in one case in which a small piece of tumor rest persisted the hormone metabolites showed no improvement even in the last control in spite of the same hormone therapy

MATERIAL AND METHOD

Seven of the eight cases of nasopharyngeal angiofibroma which were presented in 1963 were followed up and in only one of them was a piece of tumor rest found in the right corner of the nasopharynx.

The last control, of four cases out of seven was made in 1968 In these cases hormone metabolites were titrated in 24 hour collected urines (the results are given in Table 1) The excretion levels were almost normal in the tumor free cases, whereas in the single tumor-carrying case the result was still abnormal

For the titration of hormone metabolites we used again the following methods

1 Urinary 17 ketosteroids were determined by the method of Jayle Crépy & Scholler
2 17 OH(17–21 dihydroxy – 20 ketosteroid) was also determined by the Scholler Busigny & Jayle method.
3 Folliculin determination was made by the method of Jayle and his co-workers.

CONCLUSION

1 Nasopharyngeal angiofibroma is a pathology of the male

2 In the treatment of this tumor surgical removal is essential We also think that additional Testoviron therapy may be helpful

3 Following total extirpation of the tumor and additional hormone therapy hormone excretions gradually returned to normal in three patients.

4 One of the seven cases had a small tumor rest, and his hormone balance did not return to normal in spite of hormone therapy

RÉSUMÉ

Sept cas d'angio-fibromes du naso-pharynx furent discuté au congrès de 1963 du point de vue endocrinologique. Quatre de ces 7 cas, qui purent être suivi pe dant 5 ans, montrent à la dernière in estigation un tau des metabolites presque normal dan le titrage de sujets guéri de leurs tumeurs, tandis que les cas encore porteurs d tumeurs restent comme au début

ZUSAMMENFASSUNG

Beim Kongress in 1963 wurden 7 Angio-Fibroma Fälle des Naso-Pharynx om end krin logischen Standpunkt us erörtert. Bei 4 von diesen 7 Fällen die 5 Jahre la g nachuntersucht werden konnten rwiesen sich die Metabolitenwerte der letzt n Hormontitration an tumorfreien Fällen annähernd normal, an tumo haltigen Fällen dagegen blieben si fast unverändert

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S. Karatay M.D Elmek Fab Sok. 2 Sisli, Istanb l, Turkey

DISCUSSION

R Albrecht Hormonanalysen beim Nasenrachenfibrom sind äusserst schwierig, weil es sich um Personen zwischen 10–20 Jahren handelt. In diesem Alter finden wir normalerweise grosse Schwankungen der Hormonausscheidung und ihrer Metaboliten Deshalb ist es uns bisher nicht gelungen für das Nasenrachenfibrom signifikante Abweichungen nachzuweisen Bei einer Beobachtung über 5 Jahre muss man damit rechnen dass sich die hormonale Situation bei diesen Personen stabilisiert Ich habe deshalb and Hr Karatay die Frage gestellt in wieweit seine Befunde tatsächlich spezifisch auf das Nasenrachenfibrom zu beziehen sind oder auch Ausdruck des veränderten Alters der Patienten sein könnten

T Palva You have suggested that the hormonal level of 17 ketosteroids is lowered by the presence of tumours and that there is a slow return to normal after the tumour has been removed I should like to know whether or not you have observed any differences in hormone levels in varying sizes of tumour If the tumour really affects the hormonal balance then the effect of large tumours should be much greater than that of a small tumour Also when a large tumour is removed and a small recurrence is seen later is there a difference in the hormone levels pre-operatively and at the time when the recurrence is seen?

M Portmann Je voudrais poser 2 questions. (1) Le traitement par hormonothérapie peut il éviter le traitement chirurgical et quels sont à son avis le rapport de l'hormonthérapie et de la chirurgie dans le traitement des angiofibroma du naso-pharynx (2) A-t il vu des angiofibromes guéris uniquement par l'hormonothérapie.

S Karatay (Reply) to Mrs *Albrecht* In nasopharyngeal angiofibroma we find peculiar features in hormone metabolite titration Although hormone excretions are not fixed levels in all life we still know that for normal persons there are some age groups, such as prepuberty age where folliculin is about 0–7 gamma, but in adults it is 10–25 gamma In our cases we always found folliculin very high an average of 30 gamma in smaller age-groups than the normal

To Mr *Palva* You are quite right that the finding of changes in titration of hormone metabolites depends upon the size of the tumour Besides, it should be added that the age of the patient also is very important If the patient belongs to the smaller age group the tumour is larger and the result to be obtained needs a longer period Also the tumour pieces left behind have the same influence which means that if the mass is small the hormone metabolites are on a better level, or vice versa.

To Mr *Portmann* We believe that the hormone therapy before the operation reduces the hemorrage during the operation But we do not believe that only hormone therapy will reduce the size of the tumour completely that is why we say that operation is essential. Postoperative hormone therapy will give a chance to the patient to reduce imbalanced androgen-estrogen to normal doses of male hormone because we are afraid to inhibit the normal endoctrin functions.

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A CLINICAL EXPERIMENTAL INVESTIGATION OF THE RATE OF CELL PROLIFERATION IN HUMAN MALIGNANT TUMOURS

S. B. Refsum and P. Berdal

From the Institute of Pathology and the Department of Otolaryngology Rikshospitalet, University of Oslo Oslo Norway

The investigation is based upon mitotic counts in malignant tumours f llowi g i jection of a mitotic inhibitor Between the clinical growth and the cell proliferation there is, however a considerable discrepancy which can only be explained by a very substantial cell loss. The cell loss is discussed.

In the tissue under observation all growth is caused by cell proliferation or cell division The growth of a malignant tumour is also dependent on cell proliferation, but only scattered information has been available about cell proliferation in malignant tumours in man The aim of this investigation has been to measure cell proliferation in malignant tumours in man compared to cell proliferation in normal tissues.

In growing tissues and tumours there is an increase in size, i.e a positive growth, but in the adult organism there is no increase in size, i.e. a steady state Therefore there must be a cell loss keeping pace with cell proliferation. The growth can in general be expressed as the net increase of cell proliferation over cell loss

$G = P - L$ (Evensen & Iversen, 1962)

The clinical growth of a tumour can be measured by repeated X-ray examinations of primary or metastatic lung tumours over a prolonged period f time, or by direct measurement of superficial tumours. The growth is usually measured as the time needed to double the mass, the so-called "doubling time" (Collins *et al.*, 1956) This doubling time has been found t be remarkably constant in one and the same tumour indicating that growth i exponential, i.e. doubling its size at constant intervals (Breur 1966) Between different tumours, however there is great variation, with doubling times of only 6–8 days in fast-growing tumours to 300 days or more in slow-growing tumours (Steel & Lamerton, 1966)

The rate of cell proliferation can be measured by stathmokinetic methods. Drugs such as vincoblastine or colchicine and its derivatives, so-called spindle poisons, destroy the spindl apparatus of the mitoses. In so doing, they prevent the chromosomes separating and dividing cells are arrested in

DISCUSSION

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TABLE 2. *Tumour localisation*

	No. of patients
Larynx	32
Maxilla	13
Oral cavity	11
Other sites	5

nearly double this amount of cells divide The reason may be that the wear and tear of the epithelial cells in the mouth is much greater than in the larynx. In order to replace this greater cell loss a greater cell proliferation is necessary In mucous and serous glands, which are far less exposed to mechanical traumata, the rate of cell proliferation was about one tenth of the cell proliferation found in thin epithelium.

In atypical epithelium and in carcinomas the rate of cell proliferation was somewhat higher than in hyperplastic epithelium, but the difference was not pronounced (carcinomas 11 48 thick epithelium, mouth and tongue 10)

The distribution of cell proliferation found in different areas of epithelium and tumours is shown in Fig 1 The absissa shows the rate of cell proliferation and the ordinate the number of biopsies or tumour areas counted. We can here observe three points of interest

1 In all tissues there was a wide range in the rate of cell proliferation, most pron unced in atypical epithelium and in carcinomas where there was a 40-70-f ld increase in the rate of cell proliferation from slow to fast prolif rating areas.

2 Alth ugh the average rate of cell proliferation in malignant tumours was somewhat higher than in normal and hyperplastic epithelium, many

TABLE 3. *Rate and range of cell proliferation in different epithelium and in tumours*

	Rate and range of cell proliferation per 1000 cells/h
Mucous and serous glands	*0.5 (0.2–1.0)*
Columnar and thin epidermoid epithelium (larynx/trachea)	*4.8 (0.2 14 0)*
Thick or hyperplastic epidermoid epithelium (mouth/tongue)	*10 0 (1.0–23.0)*
Atypical epithelium	*12.8 (0.5–33.0*
Carcinomas	*11.45 (0 4–42.0)*

mitosis without any influence on the number of cells about to divide (Eigsti & Dustin, 1955) A biopsy taken after a colchicine injection will therefore show an increased number of cells in mitosis in the tissue If the relative number of cells in mitosis are counted and the time between the colchicine injection and the biopsy is known, the number of cells dividing per unit of time can be calculated, i e. the rate of cell proliferation

$$P = \frac{\text{No of arrested mitoses}}{\text{Time between colchicine inj and biopsy}}$$

In contrast to clinical growth and cell proliferation, there is today no known method of measuring cell loss in a tumour directly Cell loss must therefore be calculated as the discrepancy between growth calculated on the basis of cell proliferation and actual clinical growth of the tumour

MATERIAL AND METHOD

During the last 3 years we have given an i v injection of 10 mg demecolcine (Colcemid Ciba) a derivative of colchicine but considerable less toxic to nearly every patient treated for malignancy in the ENT department of Rikshospitalet We have not observed any adverse effects

Most of the patients had more or less differentiated epidermoid carcinomas (Table 1) mostly located in the larynx, the maxilla, and the oral cavity (Table 2) The average age was 59 years

Usually 2–6 pieces of the tumour were taken from each patient and in each tumour area an average of about 5000 tumour cells were counted An average of about 23 000 tumour cells were thus counted in each patient. As many of the biopsies were covered by normal, hyperplastic, or atypical epithelium the number of arrested mitoses were also counted in these tissues. The results were expressed as the number of mitoses dividing per 1000 cells per hour

RESULTS

In the normal squamous and cylindrical epithelium of the larynx and trachea the rate of cell proliferation was about five cells per 1000 cells per hour (Table 3) whereas in the hyperplastic epithelium of the oral cavity

TABLE 1 *Tumour histology*

	No. of patients
Epidermoid carcinomas	46
Salivary gland carci mas	5
Undifferentiated tumours	5
Others t mours	5

and corrected for an exponential growth pattern, the doubling time of the malignant tumours investigated should vary between 0.8 and 10 7 days, with an average of only 2 days ($G = \ln /P$)

Although the doubling time of maxillary and laryngeal tumours is unknown, we have assumed that this doubling time does not differ essentially from the doubling time of primary or metastatic lung tumours which is found to be about 2–3 months on a erage (Breur 1966 Steel & Lamerton, 1966)

The discrepancy between clinically measured growth and doubling time calculated on the basis of cell proliferation can, therefore only be due to cell loss. In this material cell loss can be calculated as 95% or more of cell renewal in the tumour How this cell loss is brought about is unknown In theory however the following may be possible

1 *Cell loss* from tumour surfaces or to blood vessels and lymphatics.
2. Necrosis due to circulatory failure (infarction) or to toxic substances.
3. Non-viable tumour cells (lethal tumour cells) due to insufficient enzyme systems, etc.
4 Immunological cytolysis due to
(*a*) Humoral or cell bound specific or unspecific antibodies.
(*b*) The organism s normal defence mechanism against *damaged or altered* cells.

Which of these processes may take place in a particular tumour is unknown Some case histories, moreover indicate that some tumours have a cell loss considerably less than calculated above, as these tumours have an exceptionally fast clinical growth without any particular fast cell proliferation. Why cell loss may fall in some patients is unknown

Neoplastic growth must be looked upon as, on the one hand, caused by an uncontrolled cell proliferation, regardless of how fast or slow it is, and on the other hand by a very substantial cell loss.

RÉSUMÉ

L'in estigation se base sur la *résultante* des mitoses dans les tumeurs après l'injection d'un inhibiteur mitotique Une *différence considérable* entre l'accroissement clinical des tumeurs et la prolifération *cellulaire* est démontrée Cett différence est expliquée par un pert cellulaire des tumeurs.

ZUSAMMENFASSUNG

Die U tersu hungen sind uf Zählungen der Mitosen na h Injektion ei er mitosenhemmenden Substanz basiert. Ein *bedeutender* Unterschied zwischen dem klinischen Zuwachs des Tumors und der zellulären Proliferation ist *gefunden*. Dieser U terschied wird durch einen Verlust von Zellen erklärt.

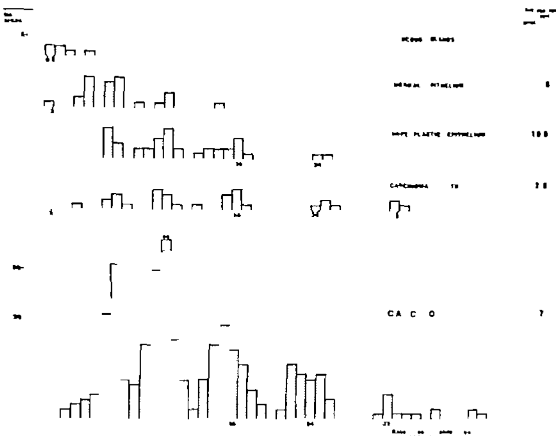


FIG. 1 Distributi n of different rates found in different areas counted in carcinomas and in atypical nd normal epithelium.

malignant tumours had a rate of cell proliferation equal to or even lower than in normal epithelium

This is of particular interest as the use of many of the cytostatica is based on the assumption of a high proliferation rate If the action of the cytostatica is solely due to its action on fast proliferating tissues, we can not expect to damage or kill tumour cells without damage to normal cells.

3 In tumours where more than one area of tumour cells was counted, different rates were often found in different areas counted. This is in good agreement with the clinical observation that a tumour does not always grow with equal speed in all areas.

Cell Proliferation and Cell Loss

As formerly stated, there is today no method of measuring cell loss directly and cell loss must be calculated as the discrepancy between rate of cell proliferation and the actual clinical growth of the tumour ($G=P-L$)

The doubling time can easily be calculated on the basis of cell prolifera tion, provided there is no cell loss. If 10 cells divide per 1000 cells per hour then all the cells would have divided in 100 hours Calculated in this way

and corrected for an exponential growth pattern, the doubling time of the malignant tumours investigated should vary between 0.8 and 10.7 days, with an average of only 2 days ($G = \ln^2/P$)

Although the doubling time of maxillary and laryngeal tumours is unknown, we have assumed that this doubling time does not differ essentially from the doubling time of primary or metastatic lung tumours which is found to be about 2–3 months on average (Breur 1966 Steel & Lamerton, 1966)

The discrepancy between clinically measured growth and doubling time calculated on the basis of cell proliferation can, therefore only be due to cell loss. In this material cell loss can be calculated as 95% or more of cell renewal in the tumour How this cell loss is brought about is unknown. In theory however the following may be possible

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Which of these processes may take place in a particular tumour is unknown. Some case histories, moreover indicate that some tumours have a cell loss considerably less than calculated above, as these tumours have an exceptionally fast clinical growth without any particular fast cell proliferation. Why cell loss may fail in some patients is unknown

Neoplastic growth must be looked upon as, on the one hand, caused by an uncontrolled cell proliferation, regardless of how fast or slow it is, and on the other hand by a very substantial cell loss.

RESUMÉ

L'investigation se base sur la résultante des mitoses dans les tumeurs après l'injection d'un inhibiteur mitotique. Une différence considérable entre l'accroissement local des tumeurs et la prolifération cellulaire est démontrée. Cette différence est expliquée par une perte cellulaire des tumeurs.

ZUSAMMENFASSUNG

Die Untersuchungen sind auf Zählungen der Mitosen nach Injektion einer mitosehemmenden Substanz basiert. Ein bedeutender Unterschied zwischen dem klinischen Zuwachs des Tumors und der zellulären Proliferation ist gefunden. Dieser Unterschied wird durch einen Verlust von Zellen erklärt.

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DISCUSSION

K H Vosteen Haben Sie auch andere Untersuchungen für Bestimmung der Malignität (z. B Chromozomenanalyse, DNS-Messungen etc.) durchgeführt und mit der Verdoppelungszeit verglichen? Wir haben versucht mit der Messung der Verdoppelungszeit während der Rö-Bestrahlung auch die Radiosensibilität eine Verdoppelungszeit von 6 Tagen nach 1000 R von 16 Tagen und nach 3000 R von 100 Tagen Ich möchte deshalb fragen ob Herr Refsum ähnliche Erfahrungen gemacht hat

S B Refsum (Antwort) an Hr *Voosteen* Wir haben Chromozomenanalyse in vielen Tumoren durchgeführt, aber wir haben noch nicht die Resultate von der Chromozomenanalyse mit der Zellproliferation verglichen Wir haben auch die Zellproliferation in Rö-bestrahlten Tumoren untersucht Die durchschnittliche Zellproliferation nach Rö-Bestrahlung war etwas weniger als die Zellproliferation in nicht Rö-bestrahlten Tumoren Der Zeitraum zwischen dem Anfang der Symptome und der Behandlung war jedoch einige Monate länger in den Rö-bestrahlten Tumoren als in den nicht bestrahlten Fällen Es ist deshalb möglich, dass die langsamere Zellproliferation in den Ro-bestrahlen Tumoren mit einer Auswahl von den Rö-bestrahlten Tumoren zusammenhängt und nicht mit einer Depression der Zellproliferation von Rö-Bestrahlung. Der Zeitraum zwischen Bestrahlung und Rezidiv war kürzer in Fällen mit einer schnellen Zellproliferation als in Fällen mit langsamerer Zellproliferation.

Der Zeitraum zwischen der Bestrahlung und der Untersuchung der Zellproliferation war einige Tage bis mehrere Jahre Wir haben keine Erfahrung mit dem Verhältnis der Zellproliferation vorher und kurz nach einer Rö-Bestrahlung

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QUELQUES ASPECTS PARTICULIERS DE LA MORPHOLOGIE PAROTIDIENNE NORMALE ET PATHOLOGIQUE

F Brunetti

Clinique Ot rhi olaryngologique de l'Université d Torino Torino Italie

Quelques charactéristiques singulières de la submicroscopie de la parotide humaine n rmale et quelques aspects peu communs de la phatologique néoplastique parotid en e so t présentés. Les don ées de microscopie optique sont comparés avec celles de la microscopie électron ique.

Particulièrement riche d études et de documentations, la littérature sur les tumeurs parotidiennes a eu comme objet de ses recherches le polimorphisme hystologique et cytostructurel des tumeurs epithéliales salivaires et les hypothèses de leur dérivation hystogénétique

Classiquement on confirme spécialement pour la parotide, la nette prédominance (incidence moyenne du 0%) des ainsi dites tumeurs mixtes qui correspondent au type des épithéliomes remaniés des AA français, dont tout le monde connait la lenteur de croissance et les poussées évolu tives, en rapport aussi avec la vie génitale (retour des règles après parotidectomie chez la femme, adénome parotidien et parotidomégalie chez l'homme dans la vieillesse)

Au point de vue chirurgical en outre, est bien determiné le rôle aggravant des interventions successives (Dargent) et l'inutilité des interventions partiales.

Dans l ordre de fréquence décroissante (à peu près le 10% des néoplasies salivaires) ui ent les tumeurs muco-épidermoidales, décrites pour la première f is en 1940 par Skorpil et successivement illustrées d'une *façon* systématique par Stewart, Forte et Becker Ces tumeurs dériveraient de cellules indifférenciées qui se seraient détachées des premiers bourgeons des duits glandulaires. Pour ce type de néoplasie on a aussi constaté une grande prédominance de localisation au niveau de la parotide (90% des cas)

La morph logi de cette tumeur est variable d'une zone à l autre En effet à côté de zones ou les cellules sont claires avec noyau placé ur la membrane cellulaire Il y a des zones où les cellules ont un cytoplasme dense et des cont urs non bien définis. Dans ces zones les cellules épithéliales nous rappellent, par leurs caractères, les éléments de l epithélium de revêtement, pouvant avoir en effet une disposition stratifiée ou bien rester réunies en l compact autour des zones constitués entièrement par des cellules à cytopl me cl ir (Figs. 1 2 et 3)

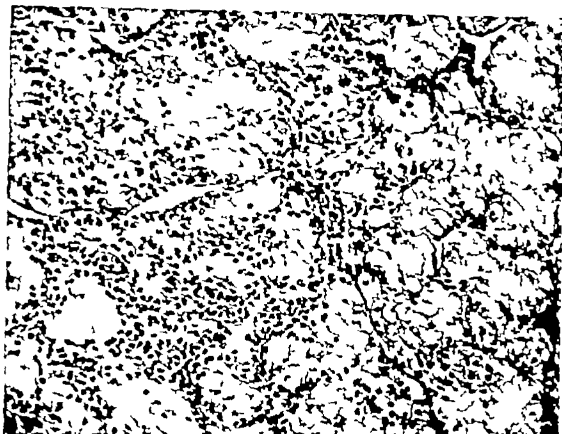


FIG. 1 Hém. éos 70 Tum ur muco-épidem Idale Vision d'ensemble la de cellules vesiculaires a co des zones d'aspect solid à citoplasm d nse (2 porti s tumorales : partie muqueuse et partie épidermoidal)

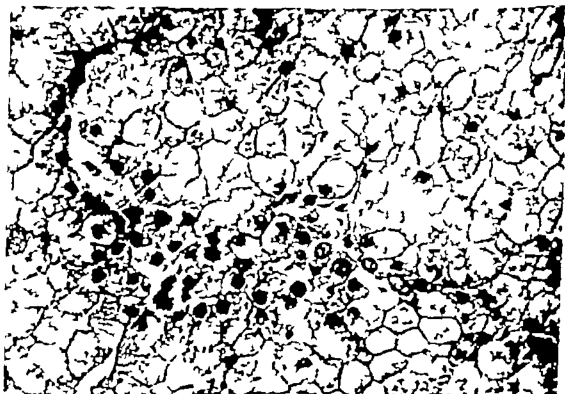


FIG. 2. Hém. éos. 3"6 Tumeu muco-épiderm Idale : Cellules écumeuses u lées avec cont urs de cell les nucléés (parti muqueuse)

FIG. 3. Hém éos. 375 T meur muco-épidermoïdal Cell les à cytoplasm dense grosses Ires da lesquelles les noy se d pose t ta hyperchromatiq es qui rappellent la morpholog des cell les d la couche basale de l'épithélium muqueux. P contre les cell les et les noy sont pl cl irs comme dans la couche muqueuse d l'épithélium (parti épidermoïdal de la t meu)



FIG. 4 McM nu 70) Adénome à cell les acineuses Résea conjoncti al qui divise des mass épithéliales cordonnées de type esiculaire

Une incidence presque analogue aux tumeurs mucoépidermoïdales aurait le cylindrome de Billroth (1859) qui a été en suite défini par Foote & Frazell (1954) comme « adenoid cystic carcinoma »

L organisation *architecturale et cytomorphologique du cylindrome*, analogue aux tumeurs mixtes, serait représentée par des formes canaliculaires, végétantes et lobulées ou par des formes acineuses et alvéolaires à cellules soit cylindro-cubiques éosinophiles, basophiles ou claires, soit fusiformes ou étoilé et enfin par des formes de dérivation qui paraissent dûes à différentes maturations ou dégénerations cytostromales (dégénération mucoïde jaline ou mixocondroïde par réaction d auto-anticorps)

Tous les éléments du carcinome adénocystique correspondent aux différentes structures cellulaires des glandes salivaires (cellule sécrétantes, bordantes et myo-épithéliales des canaux glandulaires)

Un autre type de tumeur parotidienne est l « adénome à cellules acineuses » qui dériverait des éléments des acini glandulaires

La structure générale est celle d une tumeur capsulée à tissu condensé et à caractère plurifocal Les cellules tumorales se trouvent dans des espaces allongés dont la structure est soulignée par l abondant réseau conjonctival (Figs. 4 et 5)

La bénignité hystologique apparente de cette tumeur peut être caractérisée par un décours clinique malin (possibilité de métastases à *distance* et de métastases lymphatiques régionales) et c est pour cela qu on conseille dans ces cas la parotidectomie totale avec évidement radical du cou

L adénome oncocytaire ou oncocytome est caractérisé par des cellules à cytoplasme clair éosinophile avec petit noyau à cromatin épaisse disposées en longues colonnes avec une nette et bien précise contraposition des noyaux en deux files contigües La parenchime néoplastique est divisé en lobules de dimensions inégales au centre desquels on peut observer par des phénomènes de nécrose une dissociation des cellules tumorales (Figs. 6 et 7)

L hystogénèse de l oncocytome a été attribuée à des cellules salivaires vieillissantes. Au point de vue hystogénétique les cellules de l oncocytome repètent la morphologie et les caractéristiques de l oncocyte décrit en 1931 par Hamperl et identifié comme une cellule salivaire vieillissante (Fig 8)

Fréquence mineure (4–5%) auraient enfin les adénolymphomes.

Ce type de neoplasie est constitué par des tas de cellules analogues aux éléments des conduits glandulaires, tas qui sont quelques fois réunis en longues colonnes autour de cavités d amplitude variable Dans d autres zones il y a une très riche infiltration d éléments de la série lymphocytaire qui soulèvent ces colonnes cellulaires et donnent origine à des formations papillaires, qui plongent dans des cavités de substance amorphe (Figs. 9 et 10)

L'hystogénèse la plus probable des cysto-adéno-lymphomes est représentée par la dérivation de l épithélium des conduits glandulaires excréteurs.

Sur la base des documentations récentes de la microscopie électronique

Fig. 5. Mella us 0 Adénome à cellules acineuses Di position orliqueuse du réseau conjonctif. I.

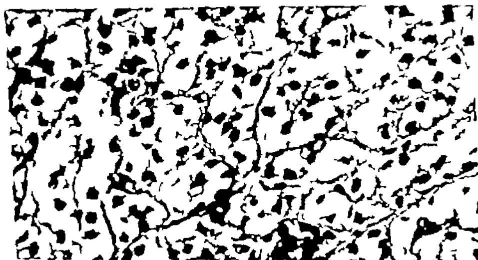


Fig. 6. M Mann 600; Adénome à cell les acineuses Morphologi typiqu d la t meur à cell les acineuses (cellules claires ec cytoplasme esleulair Pas-négatif, à di position pseudosyncitiale Il git de cell les clai es, ec nes po re de cromatine qui se trou presque toujours à la périphérie de la cellule Il est pas rare de trou er des résidu de cond it glandulaires qui conservent ne di position et ne struct re pl ou moins régulière

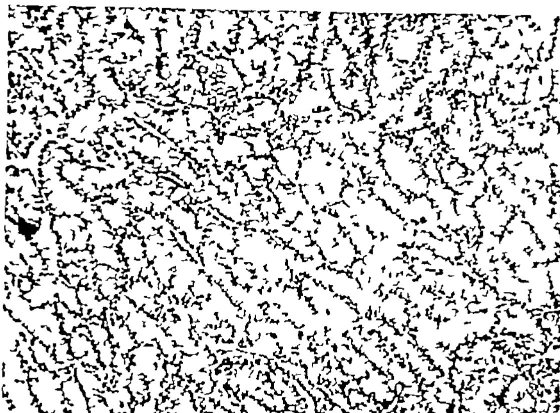
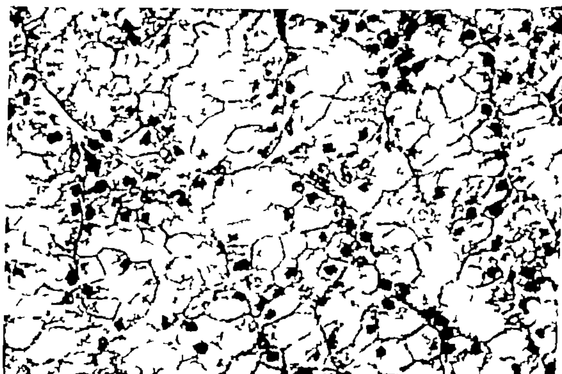


FIG. 7 Hém. éo 70 Adénom oncocytaire : Disposition à cordon des cell les à cyto-pla m granuleux et aspects pseudo-fasiculés qui s na tom sent ntr eux special ment vers la périphérie avec des ta v rtiqu ux dan l s z nes plus centrales des l bules tumoraux.



Fi 8 H m 375 Adén m oncocyt ire Caractéristiques des neoc tes cellules é. m u à l mites n ttes, d t u parti t n léée t l utre nu léée ec di po i- t n m rginal des n au



Fig. 9. Hém. éos. 70. Adénolymphome. Disposition à rubans de cellules épithéliales cylindriques et quelques formations initiales de cavités cystiques.

Nous considérerons maintenant tout d'abord l'architecture et la structure fine des glandes salivaires par rapport aux différentes situations morpho-fonctionnelles liées à l'activité sécrétoire.

Nous rappellerons à ce propos que la microscopie optique a ait *déjà per*mis de démontrer une diminution des grains spécifiques qui existent dans les cellules séreuses pendant les différentes phases de l'activité *sécrétoire glandulaire*. Pendant la phase de repos il y aurait une augmentation des mythochondres, de la *formation* de Golgi et de la substance chromophile (Bloom & Fawcett, *1968*).

Les recherches ultramicroscopiques *ont ultérieurement* specifié les caractéristiques des activités sécrétoires et des interréactions fonctionnelles intracellulaires représentées *par des introflections* de la membrane plasmatique du pôle basal, entre lesquelles se tassent des files de mytochondres (Bloom & Fawcett, 1968).

Pour la parotide humaine nous avons examiné l'ultrastructure de l'épithélium au niveau des adénomères, des *duits intercalaires* (ou prétermi-naux) et des duits striés.



FIG 10 Hém éos. × 375 *Adén lymphome : épithélium cylindrique soulevé en papilles avec un axe fibroco jonctival infiltré d lymphocytes.*

Les clichés qui suivent démontrent les caractéristiques de l épithélium de ces structures (Figs 11 12, 13 14 15)

Enfin dans la dernière photo (Fig 16) on document l aspect ultrastructurel des éléments du conduit strié La striature caractéristique de la zone basale de l épithélium des duits striés qu on voit au microscope optique parait être déterminée, au microscope électronique par des mytochondres tassés éntre les introflexions du plasmalemma

On a ainsi démontré par la microscopie électronique que l image caractéristiquement *striée de la partie basale du ainsi-dit « épithélium bacillaire* est déterminée par des tas de mythochondres intercalés entre les introflexions du plasmalemma

Cette dernière interprétation, déjà conçue par le passé comme hypothèse par la microscopie optique, confirme la grande variété cytostructurelle qui existe entre les différents éléments qui constituent les différentes portions des unités glandulaires de l organe salivaire

Ce polimorphisme cytostructurel, qui varie selon les différentes conditions fonctionnelles de l activité sécrétoire salivaire, pourrait, à notre avis, justifier l extrême variabilité cytomorphologique et hystopathologique qu on observe chez les tumeurs parotidiennes



Fig. 11. Photo électronique qui illustre les caractéristiques [illegible]-microscopiques d'un adénome. On observe des cellules où le cytoplasme paraît très dense et des autres qui paraissent plus [illegible] laires. Toutes les cellules contiennent des granules de sécrétion, qui ne sont pas tous parfaitement omogènes, [illegible] et des formations à opacité électronique [illegible] ariée. Dans la cellule [illegible] centre [illegible] microphot[illegible] on [illegible] réticul endoplasmatique réticulaire [illegible] et de nombreux ribosomes libres.



FIG. 12 Z n d cont t cell laire dans un dén mère Entre les ll les ll n y p une dhérence nt me mal ch que élément est séparé des utres pa espace perméabl aux lectrones, dan lequ l on it d n mbreuses digitation cytopl matiques n form de micro lli



Fig. 13. Phot électronique dans laquelle on obser ne cell le myo-épithéliale inter posée ntre membrane basale de l'adénomère et une cellul sali aire. Le cytoplasme de cett cell le possède un réticule granulaire endoplasmatique abondant et quelques micro- fil qui pénètrent da ne zone perméable électrones, itués entre la cellule myo- épithélial et la cellule salivaire



Fig 12 Zone d contact llul ire dans un dénomère Entre les cell les Il y a pas une adherence i tim mai chaqu élément est séparé des utres par espace perméabl u électrones, d ns leq l on oit d n mbreuse digitati ns cyt pla m tiq es en f rme d microvilli



Fig 13. Phot électronique du laquelle on obsers ne cell le myo-épithéliale ater posée entre membrane basale de l'adénomère et ne cell le saliv ire. Le cyt pl m de cette cell le possède réticul granulaire endoplasmatiq abond t et qu lq es micro-illi qui pénètrent dan ne one perméable électrones, située entre la cellule myo-épithéliale et la cell le sali aire

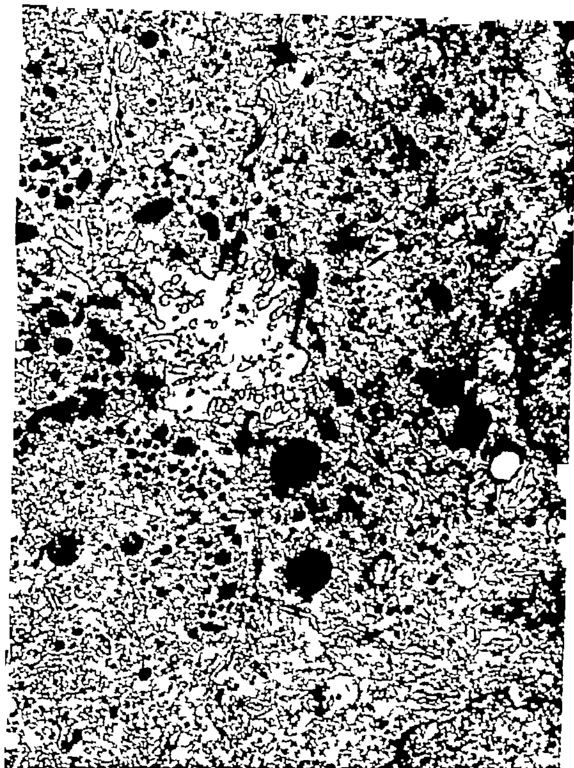


Fig. 14. Caractère submicroscopiques d l première parti d'un d it intercal ire près d l dén mère On bserve n mbreuses cellules allv ires, qui contien t plu le rs gral d sécretion, réunies nt lles pa des compl xes d j cti n à l partie plu superfi i ll Ces cellules so t nt ur d une petit ne perméabl ux électro da s laqu ll péch nt des propages cyt pla matiqu s, type micro illi et ers lesq lles sembl t se diriger les grain d sécréti

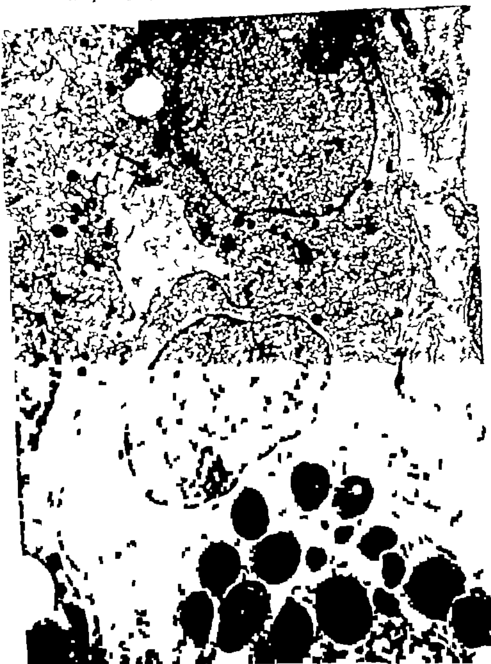


Fig. 15. Caractères submicroscopiques de la partie terminale d'un duit intercalaire vers le duit strié. Les cellules qui délimitent le duit intercalaire contiennent des grains de sécrétion en quantité inférieure à celle qu'on peut remarquer sur la photo précédente.

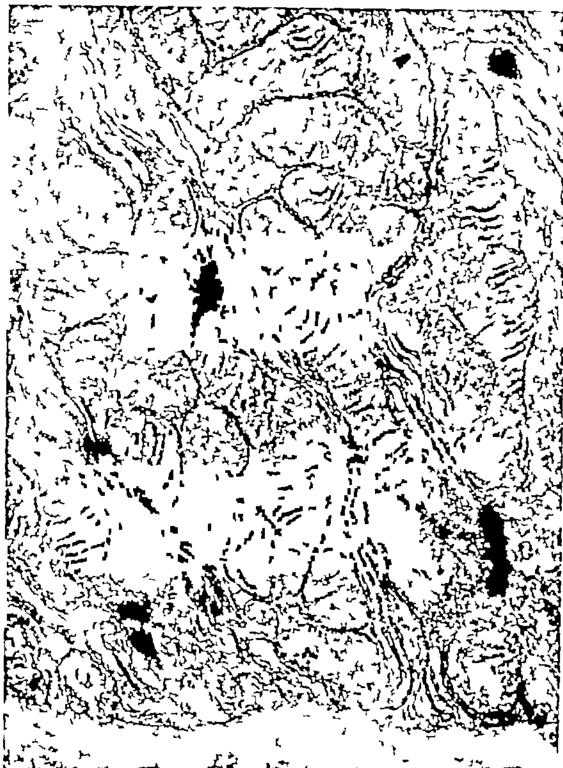


Fig. 16. Morphologie submicroscopique de l'épithélium bacillaire d'un conduit strié. La structure caractéristique de la zone basale de l'épithélium des duits striés qu... it microscope optique paraît être déterminée au microscope électronique par des mytochondres t...sés ...tre les ...trofl...xi...ns d... pl...malemma

SUMMARY

Some singular sub-microscopical characteristics of the normal human parotid and some non-common aspects of neoplastic parotid pathology are presented. Observation by optic microscope are correlated with those obtained by electro-microscopy.

ZUSAMMENFASSUNG

Verf. stellt ungewöhnliche submikroskopische Aspekt der normalen menschlichen Parotis und selte e Bilder der neopla tischen Parotispathologie dar. Die opt schmikroskopischen Bilder werde mit d nen der Elektronenmikroskopie zusammengestellt.

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DISCUSSION

J. Friedmann: As a pathologist I agree that diagnostic difficulties are encountered in this field. The surgeon is not always assisted by the pathologist s f lore arrivi g at a correct di gnosis. We have been hoping that electron microscopy might provide an additional clue. This has so far been the case only in regard to the adenolymphoma (Mr Brunetti has shown a fine example of this tumour). Light microscopy of the adenolymphoma is so characteristic that once seen, never forgotten. The electron microscope has revealed a characteristic crystalline inclusion first described by Shipke from the Sloan Kettering Institute. Two practical questions arise. The rate of incidence in B.'s material. The site of the tumours, especially the cribriform adenoma. In our material they were localised mainly in the accessory salivary glands. Differential diagnosis between "benign and malignant pleomorphocellular parotid gland neoplasms.

J. Werdill: I want to stress the importance of combining light and electron microscopy in all tumour studies. Mr Enerotth, Moberger, Hjertman, and I have studied parotid tumours with light and electron microscopy during the last few years. We have found that it is not only possible to achieve a better and more exact diagram in some cases, but also to get some information about the prognosis of the tumour by the use of this combined study.

It was found that the compact type of adenoid cystic carcinoma has a worse prognosis than the cribriform type. With electron microscopy we can prove that the prognosis is related to the amount of hyalin basilar membrane-like material produced by the tumour. High production of this substance, as in the cribriform type, is related to a better prognosis than that of tumours with a lesser production of the basement membrane-like substance.

F. Brunetti (Réponse) à M. *Friedmann* et Mr *Werdill:* Ces recherches submicroscopiques ont été faites premièrement dans la parotide normale.



FIG. 16 M rph logi submicroscopiq d l épithéli m ba ili ire d'un d it strié La tri ture caractéristique de la z ne basal d lépithéli m d d its triés qu n it a microscope optiqu parait être détermi ée u microscope électroniq par des m tochondres t sés ntre les introflexions d pl mal mma

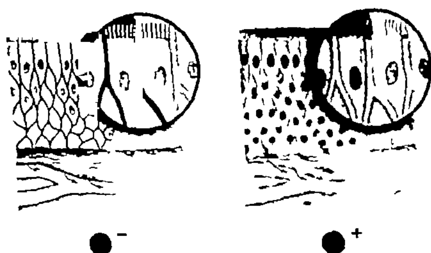


Abb. 1. Unterschiedliches Eindringen von ionisch dissoziierten kristalloiden Farbstoffen (Fluorochromen) in die Schleimhaut. *Links*: Anodische Farbstoff-Ionen werden vorwiegend *interzellulär* durch Epithel transportiert. *Rechts*: Bei kathodischen Farbstoff-Ionen erfolgt der Transport vorwiegend *intrazellulär*

Mechanismus des Resorptionsvorganges, sie erlaubten jedoch keine *quantitativen* Aussagen. Um solche zu erhalten, müssen weitere Methoden herangezogen werden. Theoretisch kommen für diesen Zweck in Frage

(*a*) Die Messung *pharmakologischer* Effekte während und nach Resorption entsprechender Wirkstoffe. Diese Möglichkeit wurde z. B. von Tonndorf *et al.* (1953) mit Hyoscamin und von Bohk (1965) mit Pilokarpin eingesetzt. Die hiermit erzielten Ergebnisse sind naturgemäß sehr grob.

(*b*) Die *indirekte Messung* radioaktiv markierter über die Nasenschleimhaut resorbierter Substanzen in den Ausscheidungen, wie sie z. B. Breuninger & Feine (1968) mit dem Nachweis von C-CO_2 in der Exhalationsluft kürzlich publiziert hat. Auch bei diesem Prinzip sind nur verhältnismäßig grobe Relativzahlen zu gewinnen, ganz abgesehen davon, daß die bisher angewandten Methoden vermutlich Fehlerquellen enthalten.

(*c*) Der *kombinierte direkte quantitative Nachweis* einer mit Nukliden markierten resorbierten Substanz am Orte der Resorption, im Blut, im Gesamtorganismus und gegebenenfalls in den Ausscheidungen. Diese Kombination verschiedener Meßverfahren gestattet nicht nur sehr viel exaktere Angaben über Art und Grad der Resorption eines Stoffes, sie erlaubt auch die Prüfung und den Nachweis von Stoffen unterschiedlichen Molekulargewichtes, sowie unterschiedlicher chemischer Verbindungen und Komplexe in systematischen Versuchsreihen.

(*d*) Prinzipiell kann diese Methoden kombination noch ergänzt werden

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ZUR PROBLEMATIK DER RESORPTIONSMESSUNG AN DER NASENSCHLEIMHAUT

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Verschiedene Methoden zur Messung der Resorptionsleistung der Nasen Schleimhaut werden kritisch diskutiert. Eigene Meßergebnisse mit J^{131} *markierten Substanzen (Jod Hippuran Lysozym, Human Albumin) sowie* kolloidalem Au^{198} werden mitgeteilt wobei verschiedene Meßverfahren kombiniert werden (Aktivitätsmessung über der Resorptionsstelle im Blut, in den Ausscheidungen sowie als Scintigramm) — Es zeigt sich, daß die gemessenen Aktivitäts Werte im Blut nicht ohne weiteres gleichzusetzen sind mit der resorbierten Menge eines markierten Komplexes (z B Human Albumin) Es gelang der Nachweis, daß von einer im Blut gemessenen Gesamtaktivität bei den Albumin Resorptionsversuchen nur etwa / auf Albuminkomplex / auf freies Nuklid oder Albumin Bruchstücke *entfallen Die möglichen Ursachen werden diskutiert und einige* weitere Fragen gestellt

Im intravitalmikroskopischen Experiment hatte einer von uns (H H. Naumann, 1958) mit Hilfe der Fluoreszenz Mikroskopie bereits früher folgendes feststellen können

1 Wasserlösliche kristalloide Lösungen werden sehr rasch von einer normalen Nasenmukosa aufgenommen Die Übergangsgeschwindigkeit ins Blut kommt unter günstigen Bedingungen der einer i v Injektion sehr nahe

2 Je nach Art der elektrischen Dissoziation der Fluorochrome ist der Resorptionsmechanismus prinzipiell unterschiedlich (Abb 1) Kathodische Ionen ( ) erfahren bei relativ langsamer Ausbreitung auf der Schleimhaut oberfläche eine *rasche* Aufnahme in die Mukosa selbst der Weitertransport erfolgt vorwiegend von Zelle zu Zelle, der Abtransport über die Gefässe ist relativ langsam Anodische Ionen ( ) hingegen breiten sich auf der Oberfläche schnell und weit aus, sie dringen etwas langsamer in die Mukosa ein und zwar *vorwiegend zwischen den Zellen, ihr Abtransport* über die Gefässe ist dann jedoch relativ rasch Nicht dissoziierte, neutrale Fluorochrome liegen in ihrem Verhalten zwischen dem der anionischen und dem der kationischen

3 Es zeigte sich ferner daß kolloidale Farbstoffe sehr viel langsamer als Kristalloide von der Schleimhaut aufgenommen werden

Diese und eine Anzahl weiterer intravitalmikroskopisch gewonnener Beobachtungen (H H Naumann, 1959) ergaben zwar *qualitative* Einblicke in den

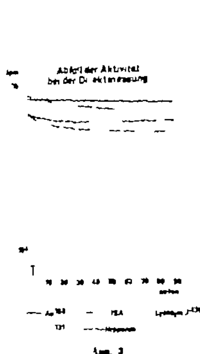


Abb. 3

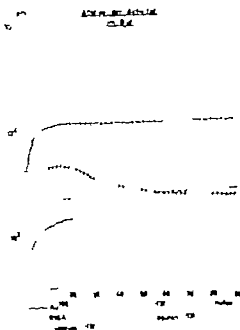


Abb. 4

gewonnenen Serum wurde dann ebenfalls mit einem Thallium-aktivierten Natrium-Jodid Kristall (Bohrloch 2 × 2 inches) die Aktivität gemessen. Abb. 4 zeigt die auf diese Weise gefundene Werte. Auf ein besonderes Problem bei diesen Ergebnissen kommen wir später noch zu sprechen.

3. Mit einem Picker Magna-Phot scanner führten wir Ganzkörper Scintigraphien an den Versuchstieren durch. Wir verglichen dabei das Scintigramm am Ende eines Resorptionsversuches mit Human Albumin (nach 90 min) mit einem Scintigramm nach einer ersten Spülung der Nase mit 100 cm³ physiologischer NaCl-Lösung, ferner einem Scintigramm nach einer derartigen Spülung und schließlich mit einem Scintigramm, nachdem alle Schleimhaut aus beiden Nasenseiten herauspräpariert worden war.

Diese Methodik bringt den Nachweis, daß bestimmte Mengen der angebotenen markierten Substanz zum Zeitpunkt der Messung in der Nasenschleimhaut abgelagert sind, daß sich jedoch auch noch freies Albumin auf der Schleimhaut-Oberfläche befindet und nicht resorbiert wurde.

4. Die Messung der Aktivität in den Ausscheidungen war im Rahmen der verwendeten Substanzen und Nuklide nur beim Hippuran interessant, da dieses Modell für ein Dipeptid bekanntlich stark harngängig ist. Die Aktivität im Blasenharn war dementsprechend bei Hippuran-Versuchen sehr hoch. Wir verzichten hier auf die Wiedergabe der Meßwerte.

Abb. 5 zeigt nun eine Gegenüberstellung der lokal gemessenen Aktivitäten über der Nasenschleimhaut und der gleichzeitig gemessenen Aktivitäten im

Zusammenstellung der untersuchten Substanzen

Aufgetragene Substanz	Größe der Substanz
J^{-131}	—
Hippuran–J^{-131}	~400 Molekulargewicht
Lysozym–J^{-131}	~14 000 Molekulargewicht 50 70 Å Teilchengröße
RISA (J^{-131} markiertes Human-Albumin)	~69 000 Molekulargewicht 150 38 Å Teilchengroße
Kolloidale Losung von Au 198	109 145 Å Teilchengroße

Abb 2 Zusamm nst llung der untersucht n Substanz n

durch *autoradiographische* Untersuchungen der Schleimhaut wahrend und nach der Resorption Unsere diesbezuglich laufenden Versuche sind jedoch noch nicht so weit ausgereift als daß sie hier zur Diskussion gestellt wer den konnen

Das oben erwähnte Prinzip der Kombination verschiedener Direktmessungen wurde von uns in den letzten Jahren ausgebaut und an einer Reihe von Substanzen mit unterschiedlichem Molekulargewicht angewendet Von einigen der dabei erhaltenen Ergebnissen soll jetzt die Rede sein

Versuchstier war aus praktischen Gründen das Kaninchen

Die Abb 2 gibt eine Übersicht über die bisher geprüften Substanzen Als Nachweisverfahren wurden die folgenden in der Isotopentechnik erprobten Meßverfahren eingesetzt

1 Die Aktivitätsmessung uber dem resorbierenden Schleimhaut Areal Sie erfolgte während einer durchschnittlichen Versuchsdauer von 90 min mit einem in 3 cm Abstand von der Nasenaußenfläche angebrachten bleiabgeschirmten Scintillations Detektor nachdem ein definiertes Volumen der zu prüfenden Substanz (10 mm³) auf die vordere Septumhälfte — bei seitlich liegendem Kopf — aufgebracht worden war Da wegen des Selbstreinigungs apparates der Mukosa (Cilien + Schleimfilm) die Gefahr besteht daß evtl. Testsubstanz uber die Choane abtransportiert und damit an anderer Stelle des Atem oder Verdauungstraktes resorbiert werden konnte, wurde der Nasenrachen der Versuchstiere mit Tamponade dicht abgestopft und bei Versuchsende die Aktivitat auch in dieser Tamponade separat gemessen. Sie betrug dort maximal 2–5% der aufgebrachten Gesamt Aktivitat Abb 3 zeigt einige Ergebnisse bei verschiedenen zur Resorption angebotenen markierten Substanzen

2 Zur Messung der resorbierten markierten Substanzmengen wurde deren Konzentration im Blut bestimmt Über die V Jugularis der dem Auftragung ort gegenüberliegenden Körperseite wurde ein Herzkatheter geschoben und in bestimmten zeitlichen Abständen je 5 ml Blut entnommen Im daraus

sprechend besserer Resorptions-Erwartung, unsere Meßerergebnisse ver fälscht haben könnten.

Zur Klärung des Sachverhaltes bot es sich an, im Jeweils bei den Human-Albumin-Versuchen gewonnenen Serum eine Trennung zwischen eiweißgebundenem J^{131} und freien J^{131} Ionen vorzunehmen. Wir verwendeten vier verschiedene Methoden, von welchen sich aber nur zwei bewährten.

Nur von diesen beiden letzteren Nachweis-Methoden soll kurz die Rede sein.

(a) Die Filtration des Serums unter Druck durch ein Eiweiß-Membranfilter (Sartorius).

Methodik. Das Filtermaterial dieser Membranfilter besteht aus Zellulose-Derivaten und anderen Polymer Stoffen und zeichnet sich grundlegend durch seine strukturelle Gleichmäßigkeit vor anderen Filter Medien aus. Wir werwendeten das Membranfilter SM 12136, für Eiweiß-Stoffe bis zu einem Molekulargewicht von 10 000. Die Filtration von 20 cm Serum erfolgte in einem Druckfiltrationsgerät (Sartorius MD 60) unter gleichmäßigem Überdruck von 10 atü. Die Aktivität von Filtrat und Rückstand auf dem Filter wurden getrennt bestimmt.

Bei diesem Verfahren zeigt sich, daß im Blut Serum in dieser Versuchsreihe nur 20% echtes mit J^{131} markiertes Human-Albumin nachzuweisen ist, während 80% der Serum Aktivität freiem J^{131} evtl. auch markierten Eiweiß-Bruchstücken mit einem Molekulargewicht von weniger als 3 000 entsprechen.

(b) Bei dem zweiten Verfahren, der Sephadex-Gel Filtration, wird das zu prüfende Serum auf eine 50 cm hohe Sephadex Säule gefüllt. Sephadex ist ein modifiziertes Dextran, ein sog. Molekular Sieb.

Die Polysaccharidketten des Sephadex sind zu einem dreidimensionalen Netzwerk verknüpft. Das stark hydrophile Gel hält Ionen zurück und läßt z. B. Eiweiß-Stoffe passieren. Wir verwendeten Sephadex G-75 normal, welches einen Fraktionierungsbereich für Peptide und Proteine zwischen 3 000 und 70 000 besitzt.

Das Filtrat wurde im Fraktionssammler aufgefangen und seine Aktivität gemessen. Abb. 6 zeigt das Ergebnis dieser Versuchsreihe. Während eine — nicht dem Resorptionsvorgang unterworfene — RISA Lösung (Kontrolle) rasch mit ihrer eiweißgebundenen J^{131} Fraktion das Gel passiert, ist nur eine verhältnismäßig kleine Fraktion von frei m J^{131} nachweisbar. — Fast umgekehrt verhält es sich bei dem Serum, das bei entsprechenden Resorptionsversuchen mit RISA aus dem Blut der Versuchstiere gewonnen wird. Hier ist der Anteil des an Eiweiß gebundenem J^{131} nur etwa 20%. Ihm stehen etwa 80% freies J^{131} gegenüber. Dies ent pricht dem Ergebnis, das über ein Eiweiß-Membranfilter gemessen wurde.

Hieraus ergibt sich:

1. Aktivitätsmessungen im Blute können bei Resorptionsversuchen irreführend sein. Es ist jeweils zu prüfen, ob der *ganze* zur Resorption angebo-

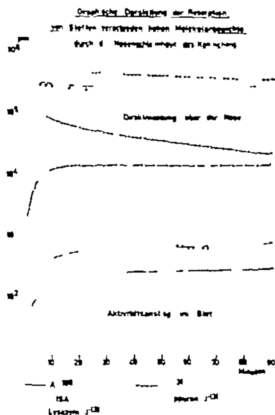


Abb. 5

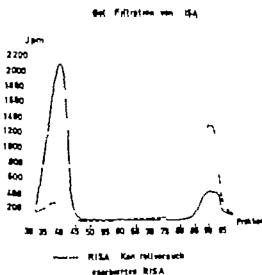


Abb. 6.

Abb. Geg nuberstelln g der Aktivität kurven: *ben* bei Direktmessung über der Nase *unt n* bei Messung im Blut

Abb. 6 Seph dex Filtrati n n ma kiert m Human-Album (RISA) Resorption und ---- markiert m Hum n Albumin, w lches über di Schleimhaut reso biert wor d n wa M n erkennt beim „nati n" Albumin den hohen Anteil an komplett m Albumin-K mplex (Frakti n 30–45) und den g ringen Ant il n frei m N klid (Fr ktion 80–95) Umgek hrtes V rhalt n bei „resorbiertem Albumin"

Blut Auf einen einfachen Nenner gebracht, zeigt sich daß J^{131} sehr rasch, kolloidales Gold jedoch überhaupt nicht meßbar im Blut aufgenommen wird Zwischen diesen beiden Extremen liegt abgestuft das Verhalten der anderen Substanzen mit unterschiedlichem Molekular-Gewicht, wobei die Resorptionsrate/Zeiteinheit umgekehrt proportional zum Molekulargewicht zu sein scheint — Dieses Ergebnis entsprach unseren theoretischen Erwartungen Bei kritischer Betrachtung schien uns aber die Aktivitätskurve im Blut für Human Albumin (RISA) mit einem Molekulargewicht von etwa 69 000 suspekt Nach dieser Kurve müßte es nämlich trotz des hohen Molekularvolumens anfangs zu einem verhältnismäßig sehr raschen Übertritt ins Blut gekommen sein Es entstand der Verdacht daß die mit J^{131} markierte Test ubstanz entgegen der allgemeinen Annahme vielleicht nicht stabil inhomogen und/oder verunreinigt sein konnte sodaß möglicherweise markierte Bruchstücke des Gesamt Eiweiß-Komplexes oder sogar das Nuklid selbst Partikel also mit sehr viel kleinerem Molekulargewicht und dement

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Hieraus ergibt sich:

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lene markierte Komplex im Blut messend nachgewiesen wird oder nur markierte Bruchstücke oder gar isolierte Nuklide

2 Es laßt sich nachweisen daß Human Albumin von der Nasenschleimhaut aufgenommen wird Seine Menge entspricht jedoch nicht der im Blut gemessenen Aktivitätskurve In der Gesamtbilanz ist von einem definierten auf die Mukosa aufgetragenem Human Albumin nach 90 min Versuchsdauer nur etwa 1% Aktivität im Blut nachweisbar Von diesem 1% ist jedoch nur etwa 1/5 eiweißgebundenes J^{131} also Human Albumin. 4/5 der Aktivität sind entweder durch freies J^{131} oder durch bis jetzt nicht naher definierbare markierte Eiweiß-Bruchstücke mit einem Molekulargewicht von weniger als 3 000 verursacht.

3 Es ist die Frage noch völlig offen, ob diese im Blut nachweisbare Differenzierung in echtes markiertes Eiweiß und Eiweiß-Bruchstücke bzw freies Nuklid lediglich durch eine Filterwirkung der Schleimhaut bei der Resorption zustande kommt ob sie bereits die Folge einer spaltenden Enzymtatigkeit in der Mukosa — also nach stattgehabter Resorption — darstellt oder ob etwa die Gefäßwände in der Schleimhaut für eine filternde Trennung verantwortlich sind

4 In bestimmten Grenzen ist die Resorptionsrate in der Zeiteinheit anscheinend indirekt proportional zum Molekulargewicht der zu resorbierenden Substanz.

5 Auf eine sehr interessante Feststellung ist noch hinzuweisen Die Partikelgröße von kolloidalem Gold ist rund 150 Å im Durchmesser die von Human Albumin 150 × 38 Å Die Partikel beider Substanzen liegen etwa in der gleichen Größenordnung Nach unseren bisherigen orientierenden Messungen werden beide Substanzen in geringem Umfang in die Schleimhaut aufgenommen Während jedoch Albumin dann auch im Blut nachweisbar wird ist dies bei kolloidalem Gold nicht der Fall Hier muß also außer der Partikelgröße noch ein anderer wesentlicher Faktor die Resorptionsrate mit bestimmen

Aus diesen Feststellungen ergeben sich weitere Fragen, so z. B

(*a*) Human Albumin (Teilchengröße 150 × 38 Å) wird von der Mukosa resorbiert und ist im Blut nachweisbar kolloidales Gold (Teilchengroße ~ 150 Å) erscheint nicht im Blut Wo liegt die kritische Teilchen-Größe für die Aufnahme in die Nasen Mukosa? Welches ist die kritische Große für Partikel, die — von der Mukosa resorbiert — noch die Wand der Schleimhaut Gefässe passieren konnen? Welche Mechanismen oder Prinzipien in der Schleimhaut sind für diese Selektion gleichgroßer Partikel in blutgängige und nicht blutgängige Substanzen verantwortlich?

(*b*) Es ist nachgewiesen (Chevance, 1957 H H Naumann, 1958 Strömme 1952) daß von sensibilisierter Schleimhaut noch sehr viel größere Partikel als kolloidales Gold aufgenommen werden können, z. B Pollen (Roggenpollen z B 30 μ) Über welche Mechanismen ist dies möglich? Handelt es

sich hierbei noch um Resorption oder etwa um eine aktive Leistung der eindringenden Partikel

(*c*) Wo speichert die Mukosa resorbierte Substanzen und was geschieht weiter mit ihnen?

(*d*) Wie verhält sich der Resorptionsvorgang, wenn entweder die physikalischen oder chemischen Eigenschaften der zu resorbierenden Stoffe abgewandelt werden oder andererseits der Zustand der Mukosa selbst verändert wird?

RÉSUMÉ

Il y plusieurs méthodes pour établi l résorption de la muqueuse nasale. Il y des difficultés considérables quant à l'd tification de substance radioactives dans le sang resorbées par l muqueuse. La dimension d la radioactivité trou ée dans le sang et d n le erum n correspond pas forcément à la substance radioactiv ppliquée à la muqueuse sale. Par conséquent nous ten n à la comparaison des substances trouvées d le sa g vec les subst ces appliquées à la muqueuse nasale suivant des méthodes de recherche qui permettent cette comparaison.

SUMMARY

Several methods for m asuring th resorption capacity of th nasal mucous membrane are critically discussed. The e perimental results communic ted in this paper have been btained by l le J[131] and J[125] traced substances (hippuran, lysozyme human albumin) as well as b colloid l Au[198]. Differe t rangi gs have been combined (ctivity computing ( ) on the region of resorption (*b*) in the blood, ( ) in th excretions, a d (*d*) by scintiscanning) A could be proved the computed activities from the blood are ot the same a those from th whole traced comple being resorbed (i.e. human albumin) We could demonstrate by our experiment on album e esorpt o that th t tal acti ity computed from the blood had been spl t up int albumine-compl x and free iodin or fragmented lbumine Possible causes for this phenom n n are discu sed

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DISCUSSION

T Palva We know from some English studies on animals that various substances instilled into the nose are taken up with the lymph vessels and they enter the circulation in this way Has Mr Naumann made any studies in this respect and what is his opinion of the part played by the lymph vessels and the capillaries in the absorption?

My second question has to do with the effect of parasympathetic and sympathetic stimulation in the nasal mucosa. Since the former causes a swelling of the mucosa and the latter a constriction of the blood vessels and mucosa, the effect on absorption could also be very much different. Is it possible that the substances introduced into the nose by Mr Naumann could as such, also cause damage in the normal basic nasal mucosal balance?

G F Greiner Je voudrais avoir l opinion de Mr Naumann sur la résorption de la molécule S par la muqueuse nasale Question qui se pose pour l'activité des Eaux Thermales

H Engström I am especially interested in the behaviour of his radioactive gold particles. In studies carried out by me and my colleagues, it has been found that different colloidal silver chemicals are taken up in a very different way although they should be very similar in molecular size From the literature we know that colloidal silver for some reason electrical chemical or other has a pronounced affirmity for the olfactory sensory cells. There seems to be a difference between the uptake of different chemicals between supporting and sensory cells. Have you in the olfactory region of the nasal mucosa, seen any difference in uptake between supporting cells and olfactory cells? Have you seen a very high uptake in sensory cells or neurol elements?

H H Naumann (Antwort) an Hr *Palva* Die angegebenen Substanzen haben wahrscheinlich keinen Effekt auf das vegetative Nervensystem. Es ist zu erwarten dass die Resorptionsrate sich ändert wenn die Mukosa unter sympatischer oder auch parasympatischer Stimulation steht. Unsere diesbezügliche Experimentalreihe ist noch nicht abgeschlossen. Wir sind z Z. dabei, auch die Aktivität in der Lymphe der Nasenschleimhaut zu messen. Dies macht noch gewisse methodische Schwierigkeiten Allem Anschein nach sind Lymphkapillaren für Partikel grosser Molekülgewichte besser durchgängig als entsprechende Blutkapillaren

An Hr *Greiner* Kolloidaler Schwefel wurde vor uns noch nicht in die Versuche einbezogen

An Hr *Engström* Differenzierte Untersuchungen darüber ob sensorisches oder respiratorisches Epithel oder Stützzellen unterschiedlich beim Resorptionsvorgang mitwirken, haben wir nicht angestellt. Vermutlich sind für die Resorption nicht nur die Partikelgrösse, sondern auch Faktoren wie die elektrische Ladung, der isoelektrische Punkt etc. wichtig so dass Unterschiede der verschiedenen Epithelarten bei der Teilnahme am Resorptionsvorgang denkbar sind

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EXPERIMENTAL TRANSFER OF ALLERGIC INFORMATION IN LABORATORY ANIMALS

S. PODVINEC, R. ANDRIC, V. SAVIC, K. MITROVIC and M. ANOJCIC

From the Clinic of Otolaryngology Medical Faculty University of Beograd and the Clinic of Surgery Faculty of Veterinary Medicine University of Beograd Beograd Yugoslavia

In three series of experiments on guinea pigs the authors have transferred sensitization to ovalbumin from sensitized animal t normal animals by means of a temporary kin graft which was e planted after staying in the recipient f r 7 days. In the same way desensitizati n could also be tra sferred s an immunological information to sensitized animals. The authors conclud that sen itization a d desensitization are coded and memorized in cell elements and can be transferred t a homologous animal in the sh pe of a molecular graft which becomes incorporated int the genetic system of immunologically competent cells.

Interest in respiratory allergy is steadily increasing. It is not only centered around the purely allergic diseases like pollinosis or allergic rhinitis. It is not caused only by the fact that many diseases of the respiratory tract are the result of a combined action of allergy and infection, like subglottic laryngitis and asthma. It is more and more widely recognized that in most instances chronic infection of the air passages has allergy as its basis and can be cured successfully only if both nosologic factors are treated Urbanization and industrialization in contemporary life are responsible for an increase in the occurrence of occupati nal allergy which is a problem in many fields of industry particularly in chemical factories. Very severe manifestations of allergy to drugs can appear in the respiratory organs, such as oedema of the larynx, particularly following administration of anti biotics and certain vitamins. An ever-increasing number of patients are coming to the department of allergy in our clinic who belong to one of these f ur groups. The greatest number belong to the group of chronic infection on which the allergic terrain can be proven in 70–80 per cent of the cases.

Th theoretical studies of the problems of allergy are becoming more and mor challenging since the transplantation of organs to human beings has become a feature of the most spectacular progress in medicine The immunological processes of rejection or tolerance to a graft are touching the fundamental questions of allergic reactions.

In the course of our experimental studies we have executed three series f experiment on guinea pigs. In all instances the method to test the

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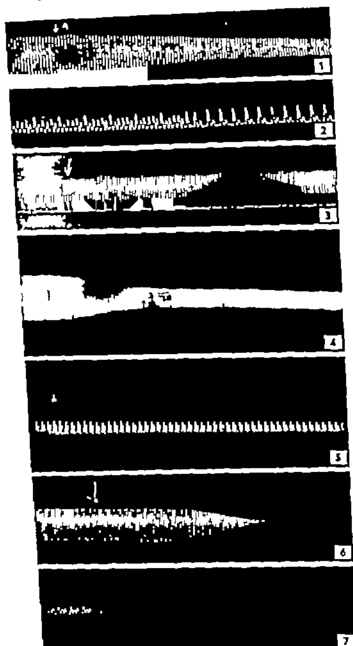
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presence of allergic systematic sensitization was performed according to the experience of Mojović & Skerović (1964) on the surviving heart of the guinea pig The animal is sacrificed and the heart is rapidly excised and fixed to the end of a tube in Langendorf's apparatus, where it can be kept beating for 20 to 30 minutes owing to a perfusion under constant temperature pressure and oxygenation The autonomous contractions of the heart can easily be registered mechanically as we did in the beginning, or with the help of a mechanotransducer as we did more recently

When the heart of an animal is examined in this way 2 weeks after intraperitoneal sensitization with ovalbumin, it will after having established regular contractions, change instantly its frequency and amplitude when a few drops of the specific antigen are added to the perfusing solution (Fig 1) After some 10 or more minutes the disturbance of the action of the heart becomes more intense and develops into an atrio-ventricular bloc (Fig 2) After some lapse of time the heart can recover again its former regular beat which shows that it was the allergic reaction, and not the trauma of the experiment, which brought about the change registered in the graph

In the first series of experiments we started with a number of guinea pigs sensitized to ovalbumin After having examined positive and negative control animals we desensitized our group with Subtivaccine This drug, won and produced on the basis of the autolysis of non pathogenic bacteria, *B Subtilis* and *B megatherium* when given to our patients has in clinical practice shown great potency in the hyposensitizing treatment of respiratory allergy As in clinical treatment Subtivaccine was employed on the surface of the skin which was scarified on the abdomen of the animals twice a week during 6 weeks. The animals were sacrificed after the end of the treatment Their surviving heart was examined by the method described When the specific antigen was added to the perfusing solution there was no perceptible reaction, the heart beat was unperturbed (Fig 3)

In the second series of experiments we tried to effect the transfer of allergic information from a sensitized to a normal animal A piece of skin was taken from the abdomen of a sensitized donor animal and transplanted to the abdomen of a normal recipient animal The full thickness graft was left in place only for 7 days and then it was surgically removed Two weeks later both the donor and the recipient animal were sacrificed and their hearts examined in the Langendorf apparatus. It was found that both the donor as well as the recipient animal reacted promptly to contact with allergen In one group of animals ovalbumin was used as antigen The graph obtained from the recipient animal (Fig 4) was no less positive than the one from the donor animal the one that had been sensitized The heart soon went into dissociation of its contractions (Fig 5) Even more pronounced and constant was the transfer of the information of sensitization when the donor was sensitized with horse serum The recipient showed intensive depression of the contraction (Fig 6) and atrio-ventricular bloc



Figs. 1 7

(Fig 7) In this second group of animals all five were equally positive, whil in the ovalbumin gro p f ur of five animals were positive.

In a third series f experiments we tried to effect the transfer of anti-allergic information to sensitized animals. Starting with two groups of guinea pigs, one sensitized with ovalbumin and the other first sensitized with o albumin and then desensitized with Subtivaccine, full thickness

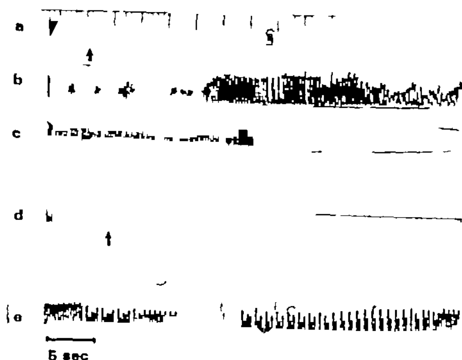


FIG. 8

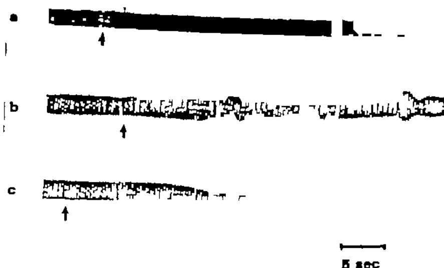


FIG. 9

skin grafts were taken from desensitized donors and temporarily implanted into sensitized recipients. In this series of experiments we repeated the observations we made in our first and second series (Fig 8) Our positive controls i e the sensitized animals, showed prompt depression of the heart beat on contact with ovalbumin (*A*) atrio-ventricular bloc some minutes later (*B*) and still later recovery (*C*) which shows the heart was not damaged in the experiment. The desensitized animals showed no reaction on contact with the antigen (*D*) Some desensitized animals used as controls showed a slight positive response to the allergen (*E*) (two animals in eight in this experiment)

In the group of sensitized recipients which had been carrying the skin from desensitized donors for 7 days and which were sacrificed 2 weeks after the explantation of the skin one half of the total number (four in eight) manifested complete desensitization (Fig. 9 *A*) Two animals of the recipient group had a high degree of desensitization (*B*) and only two were slightly positive to the specific antigen (*C*) not reaching at all the strong positive reaction of the positive controls. Of six positive controls there were three strongly positive two positive, and one uncertain. In a control group of eight animals desensitized directly with Subtivaccine two did not respond to contact with the allergen, four responded slightly and two were uncer tain.

CONCLUSIONS

1 Allergic sensitization in guinea pigs is a pattern of specific immunological reaction which is coded in cellular elements. This code of behaviour remains memorized in these cells and can be transferred to other homologous animals.

2. Desensitization and hyposensitization can be effected in sensitized animals by employing Subtivaccine on the surface of the skin by the scari fication method. Desensitization can also be coded and memorized in cell elements and can be transferred to other homologous animals.

3. The "transfer factor" first suggested by Chase (1045) then demon strated by Lawrence (1955) Lawrence & Pappenheimer (1956) and Law rence (1960) comes to evidence in our experiments. There is a possibility that the "transfer factor" is a molecular graft which becomes incorporated into the genetic system of the nuclei of immunologically competent cells and is carried over to subsequent generations of these cells, directing the pattern f the immunological behaviour of the individual

RESUME

Dan trois series d'e periences sur le cobay les uteurs ont réussi d trans mettre la sensibilisation l'ovalbumi e d l'animal sensibilisé à l' imal normal comme un transfert d'information immunologique Ils se so t servi d'une greffe cutanée dont l'animal sensibilisé était le donneur et l' nimal normal le hôt La greffe cutanée était explantée près 7 jours. On a trouvé qu'on peut transmettre la desensibilisation dans la même f çon pa un greffe à un animal homologue Les uteurs pensent que la sensibilisation et la désensibilisation sont fi ées dan la mémoire des éléme t cellulaires et que ce programme peut être transmis dans la forme d'un greffe moléculaire à un animal h mologue. Elle peut être i corporé dan le système génétiqu des cellules d ordre immun logique

ZUSAMMENFASSUNG

I drei Serien on E perimenten an Meerschwei chen ist es den Autoren g lungen eine Sens bilisierung auf Ovalbumin von den sensibilisierten Tieren of normale Tiere zu übertragen indem sie dazu ein H uttransplantat benutzten, das

nur sieben Tage im Empfänger verweilte. Auf dieselbe Weise konnte auch eine Desensibilisierung als immunologische Information auf sensibilisierte Tiere übertragen werden Die Autoren nehmen an dass Sensibilisierung wie auch Desensibilisierung als Programm in Zellelementen aufbewahrt werden und auf homologe Tiere in Form eines molekülären Transplantates übertragen werden kann Dieses kann in das genetische System immunologisch aktiver Zellen eingebaut werden.

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PODVI EC, S MOJOVIĆ M MITROVIĆ K VRČIĆEVIĆ D., nd ANDJELK IC I 1966 Recherches xpérimentales et cliniques ur la désensibilis ti n n pécifiq e dans la maladi all rgique des oies respiratoires. *Ther Umsch 23 422*

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DISCUSSION

J E Bordley Have only immediate transplants been done? Has freezing of the graft with delayed transplant been attempted? Have animals been challenged after a longer period than 2 weeks following transplant? What evidence of rejection has there been of the sensitized grafts and of the desensitized grafts? Has white blood celltyping been carried out between donor and recipient?

H H Naumann Wie wurde in der Versuchsreihe der Grad der Sensibilisierung" oder Desensibilisierung" betimmt und definiert? Wurde der Antikörper Titer im Blut festgestellt? Wurden sonstige immunologische Bestimmungen durchgeführt?

S Podvinec (Reply) to Mr *Bordley* We have used only immediate grafting which has seemed most appropriate for this work. We have never waited more than a week to explant the graft. By doing so we have not experienced the rejection of the graft though under the microscope the explanted skin has shown signs of reactions of intolerance like hyperaemia, granulation tissue and defective staining The animals used in the experiments were random samples of animals from different families.

To Mr *Naumann* Wir haben es nicht als notwendig gefunden den Grad der Sensibilizierung oder der Desensibilizierung durch humorale Messungen zu bestimmen obzwar das nicht ohne Interesse wäre Wir hielten bisher an der einen Testmethode am uberlebenden Herzen fest weil sich diese Methode als sehr empfindlich gezeigt hat und weil sie über eine längere Beobachtungszeit (bis zu 30 Minuten) ausgedehnt werden kann und sich gut registrieren lässt.

Acta oto-laryngologica 67 137 141 (1969)

RECURRENZ KURZSCHLUSS-ANASTOMOSE ZUR RÜCKGEWINNUNG DER VERLORENEN STIMMLIPPENMOTILITÄT

Kurzbericht

A Miehlke

Aus der Universitäts-Hals-Nasen-Ohrenklinik Göttingen Deutschland

E periment en an 10 Hunden wurde das Zi l gesetzt, au dem medialen Segment des Vagusstammes d n in Höh des Kehlkopfes bereits prät mierten Recurrensfasern teil abrup llen und diesen mit dem kehlkopf nahen Recurren stumpf zu anastomosieren. Nach 7 bi 10 M ten wurde der Erfolg der Reinnervation im Bereich der i neren Kehlkopfmuskula tur morph logisch bjekt iert.

Eine Arbeitsgruppe um Miehlke (Dal Ri, Schmidt, Küsel, Haubrich und Schätzle) untersuchten im Tierexperiment die Frage ob eine Reinnervation der Stimmlippen nach einer Vagus-Recurrensplastik zu erzielen ist. Dieses Problem i t von praktisch klinischem Interesse weil in ca. 0,3 (Lahey & Hoover 1938) bi 9 4 % (Blomstedt & Rydmark) nach Strumaoperationen eine iatrogene Läsion der Recurrentes stattfindet und auch in neuerer Zeit durch schwere Verkehrsunfälle immer häufiger Verletzungen der Recur rensnerven eintr ten.

Die Wied rherstellung der normalen Funktion des N recurrens wäre nach Operations- und Unfallverletzungen natürlich am einfachsten durch die primare Nervennaht, wie sie zuerst Stierlin (1916) Lahey & Hoover (1938) angaben, bei breiteren Defekten durch die Autonerventransplantation Miehlke (1967) zu erreichen.

Die Wiedervereinigung der Nervenstümpfe bei einem durchtrennten N recurrens bereitet vielfach grosse Schwierigkeiten, da es sehr schwer sein kann, den kehlkopffernen Recurrensstumpf im postoperativen Narbenge webe aufzufinden Diese Schwierigkeit gilt nicht für den kehlkopfnahen Stumpf des N recurrens, da feste Markierungspunkte sicher zu ihm hin führen.

Zu Rückgewinnung der Stimmlippenmotilität ist die morphologische Wiederherst llung des peripheren Recurrensneurons die Voraussetzung. E wurde deshalb im Tierexperiment eine sogenannte Kurzschluss-Anastomose des N recurrens gebildet. Es war das Ziel, au dem medialen Segment des N vagus den in Kehlkopfhöhe bereits präformierten Recurrensfaseranteil (Abb 1) unter dem Operationsmikroskop abzuspalten und diesen mit dem

nur sieben Tage im Empfänger verweilte. Auf dieselbe Weise konnte auch eine Desensibilisierung als immunologische Information auf sensibilisierte Tiere übertragen werden Die Autoren nehmen an, dass Sensibilisierung wie auch Desensibilisierung als Programm in Zellelementen aufbewahrt werden und auf homologe Tiere in Form eines molekülären *Transplantates übertragen werden kann. Dieses kann in das genetische System immunologisch aktiver Zellen eingebaut werden.*

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phologisch (Histologie Histochemie) sowie funktionell (direkte Laryngoskopie Elektromyographie, Zeitrafferfilm) objektiviert und im Vortrag anhand eines Filmes demonstriert.

Es konnte gezeigt werden, dass durch eine solche Vagus Recurrensplastik (Recurrens-Kurzschluss Anastomose) die echte Recurrensfunktion, wenn auch nicht vollständig, so doch in einem klinisch vielleicht ausreichen den Grade zurückzugewinnen ist

Der interessierte Leser sei hinsichtlich aller Einzelheiten des experimen tellen Vorgehens und der Auswertung der Ergebnisse auf die Originalarbeiten von Miehlke und Mitarbeitern (*Arch Klin Exp Ohr Nas Kehlkopf heilk* *188* 654–667 (1967) sowie *188* 668–680 (1967) verwiesen.

RÉSUMÉ

Chez 10 chiens on a détaché du segment médial de la souche pneumogastrique à la hauteur d larynx la partie cont nant des fibres récurre tielles déjà préfor mées t on les anastomosées sur le pédicule du récurrent à pro imité du larynx. Après 7 à 10 mois l succès de la réinnervation fut objectivé dan l domaine morphologique et fonctionnel.

SUMMARY

In experiments on dogs the part of the recurrent nerv already performed at larynx l el nd well isible in the vagus nerv was isolated and anastomised with the trunk of the recurrent laryngeal nerve near to the larynx. After a regeneration time f 7 10 month histological, histochem cal, and electromyographic xamination demonstrated that it is possible to chieve reinnervati n f the laryngeal muscles by such form of europlastic procedure

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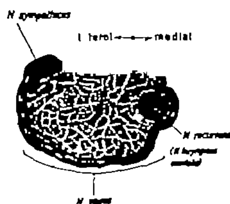


Abb. 1 Querschnitt durch den N vagus beim Hunde in Höhe der Kehlkopfregion nach Chase und Ranson 1914.



Abb. 2. Vagus-Recurrensplastik oder Recurrens-Kurzschluss-Anastomose nach Miehlke

Kehlkopfnahen (stets sicher auffindbaren) Recurrensstumpf zu anastomosieren (Abb 2)

Die Berechtigung zu einem solchen Vorgehen ergibt sich durch die eigenartige Lageentwicklung des N recurrens Bekanntlich wird dieser aus seiner ursprünglichen Lage mit einem kehlkopfnahen Abgang vom Vagusstamm beim Descensus cordis in die obere Thoraxapertur heruntergezogen Diese ontogenetisch bedingte Verlagerung wird in einigen Beobachtungen nicht vollzogen Cattel (zit nach Berendes, 1956) fand bei 479,5 Strumektomien neunmal daß der N recurrens vom Vagus in Hohe des unteren Schildknorpelrandes abging und ohne Umweg in die Kehlkopfmuskulatur eindrang und gleiche Beobachtungen liegen vor von Stedmann & Hart (1902) Lahey & Hoover (1938) sowie Frauzino Pereira (1951) Diese Rückbesinnung auf *primitive Lagebeziehungen stellte das Modell für unsere Überlegungen dar*

Die Ergebnisse unserer Recurrensplastiken an 10 Hunden werden mor

herstellung der peripheren Recurrensneurons mit Hilfe der einfachen Naht hatte ch noch nicht Gelegenheit durchzuführen. Dagegen gelang es mir vor 2 Jahren, einen Defekt im Verlauf des N. recurrens durch ein Autonerventransplantat zu überw l den. Als Spendernerven verwendete ich den N auricularis magnus. Ich möchte Ihnen hier nhand einiger Diapositive das erfreuliche Ergebnis dieser Wiederherstellung des Recurrensverlaufes zeigen Sie sehen, dass die Stimmlippenbeweglichkeit zu einen guten Teil und in ausreichendem Masse wiederhergestellt ist.

An Hr *Engström*, Hr *Arslan*, Hr *Brunetti* u d H *Angell-James* Ich danke für Ihre Bemerkungen, die zu weiterer Forschung in bestimmter Richtung anregen.

An H *Jeschek* Ich bin i der Lage, Ih en einen Querschnitt durch den N agus beim Menschen zu zeigen. Dieses Präparat wurde möglich, weil ich in einem Fall einer Neck Dissection bei Tumori vasion unter anderem in den N vagus, gezwungen war diesen unter der Schädelbasis zu resezieren. Deutlich kann man hier bereits die separierten und gebündelten Recurrensfasern im medialen Anteil des N vagus erkennen und ich konnte fi den, das die Separierung dieses Recurrensfaserbündels vom übrigen Vagus ohne jede Traumatisierung gell gt.

An Hr *Jongkees* Es handelt sich hier um Akut Experiment Es ist wichtig — und ich danke für den Hinweis — dass in weiteren Versuchsreihen die Frage des zweckmässigsten Zeitpunktes der I tervention geklärt werden sollte d. h. wi müssen in chronischen Experimenten ersuchen festzulegen, bis zu welchem Zeitpu kt überhaupt mit einer Reinnervation nach Wiederherstellung des peripheren Recurrensneurons in dieser der jener Methodik zu rechnen ist. Wir werden d rartig U tersuchungen aufnehmen.

DISCUSSION

M Portmann Avez vous une expérience de cette chirurgie chez l'homme? Car j'ai eu l'occasion durant une thyroïdectomie de faire une suture recurrentielle chez l'homme avec un excellent résultat fonctionnel final.

H H Naumann 2 Fragen (1) Wie sind die anatomischen Verhältnisse bezüglich Vagus und Recurrens-Bündel beim Menschen? Gestattet es die anatomische Situation eventuell auch beim Menschen derartige Anastomosen anzulegen? (2) Wie ist im Experiment am Hund die Funktion des Stimmbandes in Bezug auf die Synchronisation mit der gesunden Seite und in Bezug auf die Qualität der Stimme (d. h des Bellens)?

H Engström It has been shown recently by Rune Stenborg that all motor end plates disappear after a complete sectioning of the recurrent nerve The final evidence of a regeneration would be the reappearance of motor end plates. Have you looked upon this?

M Arslan The propioceptive function plays a very important part in the recurrential function Would it be possible to testify this fact in your very interesting experiments?

F Brunetti Je voudrais demander si par hasard on a essayé de faire des épreuves pharmacologiques avec les curares pour contrôler le différent comportement electromyografique des activités musculaires et adductives dans la régénération nerveuse

J Jeschek Wenn man auch Ergebnisse von Tierexperimenten nicht ohne weiteres auf den Menschen übertragen kann so haben die interessanten Untersuchungen von Miehlke wieder bestätigt dass nach Durchschneidung der Nervus recurrens eine Paramedianstellung des Stimmbandes erfolgt Dieser Befund stimmt mit unseren Ergebnissen der Nervendurchschneidungen beim Menschen überein

Dann möchte ich noch fragen, ob man beim Menschen den N recurrens ebenfalls so exakt vom Vagus abpräparieren kann Weiter haben Sie versucht im Recurrensbündel des Vagus die Abduktions und die Adduktionsfasern zu trennen? Ich glaube, dass bei einer Stenose der Glottis mit Paramedianstellung der Stimmbänder es vorallem wichtig wäre die Abduktionsfasern des Vagus mit dem distalen Recurrensstumpf zu verbinden.

L B W Jongkees Have you also been doing chronic experiments, i e graftings of the recurrent nerve some days or weeks after section of the nerve?

J Angell-James The success of this experiment may depend upon the shortening of the nerve regenerating pathway Suture of the nerve in the neck was performed in a soldier wounded by a small fragment of shell. The inner chord of the vagus nerve was cut cleanly at the level of 6th C Vertebra with immediate loss of voice At operation 3 weeks later the shell fragment was removed from the nerve in which it was embedded. The cut ends were freshened and sutured easily end to-end The voice recovered but when the larynx was examined 5 years after the war it was found that the vocal cord was still paralysed

A Miehlke (Antwort) an Hr Portmann und Hr Naumann Es ist wichtig zu wissen dass Herr Portmann ebenso wie Zöllner Doyle und andere mit der direkten Vereinigung des durchtrennten N recurrens mit Hilfe der einfachen Naht zu einer Funktionswiederkehr der Stimmlippen gelangt sind Die Wieder





Abb. 1 Abb. 2.

Abb. 1 Gaumensegelparese rechts nach thorakaler Unterbrech g on V gus und V recurren durch T mor

Abb. 2 X agus ter dem Abgang des N. recurrens durch Tumor olkommen zerstört (elßer Pf l)

der Recurrens- und Vagusausschaltung beobachtet wurde würde der experimentell nachgewiesenen Durchschneidung des N. recurrens und des R. externus des N. laryng. superior oder einer Durchschneidung des Vagus ber dem Abgang des N. laryng. superior entsprechen

Somit wäre im letzten Fall durch die totale Leitungsunterbrechung des Vagus und des Recurrens eine retrograde motorische Schwächung des oberen Kehlkopfnerven eingetreten, so daß durch Nachlassen der Spannung des Musculus cricothyreoideus eine schlaffe Intermediärstellung des betroffenen Stimmbandes eing treten ist Da es sich bei diesen Fällen um langsam wachsende Tumoren des Oesophagus und der Bronchien, um eine Struma maligna und um ein langsam sich ausweitendes Aneurisma der Aorta gehandelt hatt wurde von Hofer angenommen, daß diese retrograde zusätzliche Funktionsschwächung des Stimmbandes durch eine tigrolytische Degene r tion der Ganglienzellen im Nucleus ambiguus hervorgerufen wird. Der Sitz diese beschriebenen zusätzlichen retrograden Störung im Bereich des oberen K hlkopfnerven würde somit im motorischen Stammganglion, also bulbär bedingt sein, während der Ausfall des Recurrens peripher l kalisiert ist.

Auf der Suche nach anderen solchen retrograden Funktionsstörungen im Vagusbereich haben wir an der Grazer Klinik und an meiner eigenen Ab-

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ZUM PROBLEM DER RETROGRADEN MOTILITÄTSSTÖRUNGEN IM VAGUSBEREICH

J. JESCHEK

Leoben, Österreich

Die Beobachtungen nach Nervendurchschneidungen und Tumorzerstörungen des Vagus und seiner motorischen Äste haben erwiesen, daß nicht nur distal der Leitungsunterbrechung, sondern auch proximal derselben motorische Ausfälle auftreten können. So führten Laesionen des Vagus unter dem Abgang der Rr. pharyngici zu Paresen des Gaumenbogens und es traten schlaffe Lähmungen der Stimmbänder in Intermediärstellung auf, wenn der N. recurrens und der N. vagus unter dem Abgang des Recurrens unterbrochen waren, während bei Unterbrechung des Recurrens allein eine Paramedianlähmung auftritt. Auch nach tiefer thorakaler und abdominaler Vagotomie konnten retrograd oft wechselnde Störungen der Motilität und der Koordination der Bewegungen des Gaumensegels festgestellt werden. Es wird angenommen, daß im Vagus der Ausfall sensibler Potenzen den Tonus segmentär höher innervierter Muskeln herabsetzen kann, oder bei längerer Leitungsunterbrechung retrograde Ganglienzellendegenerationen im Nucleus ambiguus eine ähnliche Wirkung auslösen.

Unter retrograden Motilitätsstörungen verstehen wir motorische Ausfälle, die nicht distal einer Nervenlaesion, sondern proximal einer solchen auftreten. Erstmalig hat Mann (1904) die Vermutung ausgesprochen, daß die kombinierten Lähmungen von Stimmband und Gaumensegel bei thorakaler Unterbrechung durch rückläufige Degeneration der Nervenfasern und Übergreifen auf den Plexus pharyngeus bedingt sein können. Auch ein rückläufiger Reflexvorgang vom Recurrens ausgelöst, wird von ihm in Erwägung gezogen. Im Jahre 1944 konnte Hofer auf Grund von 4 obduzierten Fällen nachweisen, daß bei Zerstörung des N. recurrens und bei kompletter Ausschaltung des N. vagus unter dem Abgang des N. recurrens eine schlaffe Stimmbandlähmung in Intermediärstellung auftritt, während bei Zerstörung des N. recurrens allein ohne Vagusausschaltung (3 Fälle) eine Lähmung in Paramedianlage zu beobachten war. 1964 wurden mit Hofer gemeinsam noch weitere 7 obduzierte Fälle mit Recurrens- und Vaguszerstörung in Intermediärlähmung veröffentlicht. Die Paramedianlage bei Recurrensausschaltung entspricht den Ergebnissen der mit Hofer gemeinsam mehrfach durchgeführten operativen Durchschneidung des N. recurrens am Menschen. Die schlaffe Intermediärlähmung, die bei der Kombination





Abb. 1 — Abb. 2.

Abb. 1. ...parese rechts nach thorakaler Unterbrechung am N. ... gus und N. recurrens durch Tumor

Abb. 2. N. ... gus unter dem Abgang des N. recurrens durch Tumor ... llkommen zerstört (... eiße Pf ...)

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Somit wäre im letzten Fall durch die totale *Leitungsunterbrechung* des Vagus und des Recurrens eine retrograde *motorische Schwächung* des oberen Kehlkopfnerven eingetreten, so daß durch Nachlassen der Spannung des Musculus cricothyreoideus eine schlaffe Intermediärstellung des betroffenen Stimmbandes eingetreten ist. Da es sich bei diesen Fällen um langsam wachsende Tumoren des Oesophagus und der Bronchien, um eine Struma maligna und um ein langsam sich ausweitendes Aneurisma der Aorta gehandelt hat, wurde von Hofer *angenommen*, daß diese retrograde zusätzliche Funktionsschwächung des *Stimmbandes* durch eine tigrolytische Degeneration der *Ganglienzellen im* Nucleus ambiguus hervorgerufen wird. Der Sitz dieser beschriebenen *zusätzlichen* retrograden *Störung* im Bereich des oberen Kehlkopfnerven würde somit im *motorischen* Stammganglion, also bulbär bedingt sein, während der Ausfall des Recurrens *peripher* lokalisiert ist.

Auf der Suche nach anderen solchen retrograden Funktionsstörungen im Vagusbereich haben wir an der Grazer Klinik und an meiner eigenen Ab-

tellung durch Jahre hindurch die kombinierten Lähmungen von Stimmband und Gaumensegel beobachtet und gesammelt So konnten wir von 21 Lähmungen des N vagus, 12 Fälle kombiniert mit Gaumensegelparesen bei thorakaler Vagusschädigung gemeinsam mit Hofer im Jahre 1964 veröffentlichen (Abb 1) Bei 11 von diesen Patienten war eine einseitige Stimmbandlähmung in Intermediärstellung und eine Gaumensegelparese und bei einem nur eine Gaumensegelparese allein vorhanden Bei 4 Fällen, bei welchen bereits längere Zeit ante exitum eine einseitige Gaumensegelparese und Stimmbandlähmung in Intermediärstellung festgestellt werden konnte hatten wir auch Gelegenheit bei der Obduktion eine genaue Lokalisation der schädigenden Noxe festzustellen. Bei allen 4 Fällen war es durch destruierende Prozesse zu einer kompletten Leitungsunterbrechung im N vagus und im N recurrens gekommen Bei diesen Fällen kann somit die Gaumensegelparese nur durch einen retrograden Vorgang erklärt werden, da die Unterbrechung im thorakalen Anteil des Vagus erfolgt ist

Aber nicht nur bei solchen langsam progredienten Zerstörungsprozessen kann es zu einer solchen retrograden Motilitätsstörung kommen, sondern auch unmittelbar nach einer operativen Durchtrennung des N vagus. So hat Messerklinger (1949) einen Fall veröffentlicht bei dem vor der Operation einer malignen Struma eine normale Beweglichkeit des Gaumenbogens und der Stimmbänder nachgewiesen war und bei dem nach Durchtrennung des N vagus in Ringknorpelhöhe eine schlaffe Lähmung des Stimmbandes in Intermediärstellung und eine Gaumensegelparese auftrat Einen zweiten Fall einer kombinierten Lähmung nach operativer Vagusdurchschneidung konnte ich beobachten, nur war das Stimmband zwar schlaff auch etwas exkaviert, doch in einer Stellung zwischen Paramedian und Intermediär Die Stimme aber war hochgradig heiser fast aphonisch wie sonst nur bei der Intermediärlähmung und im Stroboskop konnte man über die Medianlinie überschlagende flatternde Schwingungen, wie wir sie nur bei vollkommen schlaffen Stimmbändern sehen, feststellen

Außer diesen beschriebenen Fällen einer retrograden Störung im Bereiche des oberen Kehlkopfnerven und im Versorgungsgebiet des Gaumenbogens nach Ausschaltung des Recurrens und des N vagus hatten wir auch Gelegenheit eine retrograde Motilitätsstörung des Gaumenbogens nach totaler Leitungsunterbrechung des N vagus allein unter dem Abgang des N recurrens zu beobachten An dem bei der Obduktion gewonnenen Präparat kann man deutlich die komplette Zerstörung des Vagus unter dem Abgang des Recurrens sehen (Abb 2) während der obere Vagus und Recurrens vollkommen intakt ist Bei dieser Patientin konnten wir bei mehreren Untersuchungen eine Gaumensegelparese rechts bis kurz vor dem Tod bei normal beweglichen Stimmbandern nachweisen Auf Grund dieses Befundes und unserer langjahrigen Erfahrungen mit solchen Motilitätsstörungen haben wir bereits vor dem Exitus eine völlige Leitungsunterbrechung des rechten N vagus kaudal vom Abgang des N recurrens angenommen

In letzter Zeit habe ich nun mit Stauber (1968) gemeinsam Gelegenheit



Abb. 2. Gaumensegelparese rechts nach vorderer trunkulärer Vagotomie.

gehabt, 14 Patienten, bei denen wegen Ulcusbeschwerden eine Vagotomie durchgeführt wurde, laryngologisch längere Zeit zu beobachten. Die verschiedenen Arten der Vagotomie erfolgten in Hiatusnähe. Einmal wurde eine beidseitige thorakale, 3mal eine beidseitige abdominale trunkuläre, 4mal eine abdominale trunkuläre vordere, 1mal eine abdominale trunkuläre hintere und 5mal eine abdominale totale Vagotomie bei Gastrektomie durchgeführt. Bei diesen Patienten wurde nun am Tag nach der Operation beginnend, und bei 6 entscheidenden Testfällen bereits vor der Operation ein genauer neurologischer Pharynx- und Larynxstatus erhoben. Bei keinem dieser Patienten konnten Motilitätsstörungen an den Stimmbändern festgestellt werden. Wohl aber wurden bei 13 Fällen oft wechselnde Motilitätsstörungen des Gaumenbogens bei Phonation, bei Respiration und beim Würgreflex beobachtet. Bei 8 Fällen war eine Asymetrie der Gaumensegelbewegung vorhanden, die bei aufeinanderfolgenden Untersuchungen einmal nach rechts und einmal nach links gerichtet war. Manchesmal sahen wir kreisende Bewegungen, so daß das Gaumensegel einmal nach der einen und dann nach der anderen Seite verzogen wurde. In diesen Fällen haben wir es vermieden von einer Parese zu sprechen, sondern uns darauf beschränkt, die jeweilige Seitenverschiebung bekannt zu geben. Es dürfte sich dabei um wechselnde retrograde Tonusherabsetzungen der Gaumenmuskulatur und zum Teil um Koordinationsstörungen der Innervation des beidseitigen Einsatzes handeln. Bei 5 Patienten aber sahen wir eine bleibende, zum Teil mehrere Wochen lang beobachtete Seitenverschiebung. Wir

sind der Meinung daß wir in diesen Fallen von einer partiellen Parese sprechen konnen (Abb 3) Wir fanden 2 Paresen des rechten Gaumenbogens bei totaler Vagotomie eine Parese des linken Gaumenbogens und eine Parese des rechten Gaumenbogens bei vorderer trunkularer Vagotomie und schließlich eine Parese des rechten Gaumenbogens bei der einzigen von uns durchgeführten hinteren Vagusdurchtrennung Diese sicherlich nur retrograd zu erklärenden Bewegungsstörungen des Gaumenbogens treten nun nicht wie bei den distalen Lahmungen in der Regel immer an der Seite des unterbrochenen Vagusanteiles auf eine Beobachtung die auf Grund der anatomischen Struktur des N vagus im Hiatusbereich leicht zu verstehen ist. Denn beide Vagi bilden ober dem Hiatus einen Plexus mit zahlreichen Anastomosen untereinander Die Äste des linken Vagus mit den Zweigen des rechten vereinigen sich zu einem Truncus, der an die Vorderseite des Oesophagus zieht während auf gleiche Weise gebildet der Truncus des rechten Vagus an die Hinterfläche des Oesophagus gelangt Somit haben beide Trunci in Hiatusnahe Nervenfasern vom rechten und linken Vagus. Eine Seitenbeeinflussung des Gaumensegels würde also von der individuellen Variante der Anastomosenbildung abhängen Schwer zu erklären wäre nur wieso bei 3 totalen Vagotomien nach Gastrektomie 3mal eine Parese des rechten Gaumenbogen aufgetreten ist.

Eine beiderseitige Einschrankung der Gaumenbogenbeweglichkeit sahen wir 5mal 3mal bei Gastrektomie und beidseitiger Vagotomie entsprechend der beidseitigen Nervenstorung 1mal bei einer vorderen und einmal einer hinteren trunkulären Vagotomie kombiniert mit einer Seitenverschiebung

Eine weitere Form der Motilitätsstorung, die wir sonst nur bei zentralen Ausfällen im Vagusbereich zu sehen gewohnt sind, wurde bei 4 Fällen beobachtet, namlich eine Koordinationsstörung der automatisch gesteuerten Gaumensegelbewegungen bei Ein und Ausatmung, bei Phonation und beim Würgreflex. Man hatte den Eindruck als wüßste das Gaumensegel nicht mehr wie es sich bei seinen verschiedenen Funktionen zu verhalten hat So wurde z. B das Gaumensegel in zwei Phasen bei der Phonation kontrahiert, blieb dann aber auch bei der Atmung in dieser Phonationsstellung und es kam erst zur Entspannung wenn der Mund geschlossen wurde Diese eigenartigen Koordinationsstörungen betrafen auch die respiratorischen Bewegungen Während normal bei der Inspiration das Gaumensegel gesenkt und bei Exspiration gehoben wird traten gegenteilige paradoxe Bewegungen auf Bei 7 Fallen wurden Zuckungen des Gaumenbogens bei Phonation wie auch bei Entspannung beobachtet

Aber nicht nur im motorischen Bereich sondern auch im sensiblen wurden bei der tiefen Vagotomie retrograde Beeinflussungen festgestellt So fanden wir bei 10 von 14 Fällen eine Verminderung des Würgreflexes. Diese Tatsache ist vielleicht am leichtesten erklärbar da der sensible Anteil des Reflexbogens, in dem die Cardia und die Organe des Abdomens liegen und in dem auch der Gaumenbogen einbezogen ist unterbrochen wurde Nervöse Schluckstörungen wurden nicht beobachtet

Diese erwähnten motorischen und sensiblen Ausfallserscheinungen waren in den ersten Tagen nach der Operation am stärksten ausgeprägt, zum Teil veränderten sie sich, zum Teil nahmen sie nach wenigen Wochen ab, oder verschwanden. Bei 2 Patienten, bei denen allerdings keine Voruntersuchung stattgefunden hatte, waren auch nach 20 Monaten bzw. 3 1/2 Jahren deutliche Abweichungen der Gaumensegelmotorik zu sehen.

Die Tatsache, daß bei solchen thorakalen und zum Teil abdominellen Vagusausschaltungen segmentär höher gelegene Nervenäste funktionelle Ausfälle aufweisen, ist wohl auf Grund dieser genau lokalisierten Nervendurchschneidungen und zum Teil auch autoptisch verifizierten thorakalen Destruktionsprozessen nicht mehr abzuleugnen. Es fragt sich nur, wie man diese Beobachtungen erklären kann. Um diesem retrograden Phänomen näherzukommen, müssen wir berücksichtigen, daß der N. vagus kein rein motorischer Nerv ist, sondern afferente sensible Fasern, afferente sensorische (Geschmacksfasern), efferente motorische Fasern, sympathische und schließlich autonome dem Parasympaticus angehörige Neuriten enthält. Da außerdem in den Kehlkopfmuskeln von verschiedenen Autoren (Brocklehurst & Edgeworth, 1940; Goertiler 1950; Paulsen, 1958; König & von Leden, 1961; Lucas Keene, 1961; Rudolph, 1956) Muskelspindeln nachgewiesen wurden, von Mollnari (1962) auch elektrophysiologisch bestätigt werden konnte, muß auch das System der efferenten kleinkalibrigen Nervenfasern vorhanden sein, das den Grad der tonischen Spannung bestimmt, indem es die für die reflektorische Tätigkeit entscheidende afferente Entladung der Muskelspindeln moduliert. Der Tonus im Warmblütermuskel wird vom Zuckungssystem der großkalibrigen motorischen Fasern *derart* hervorgerufen, daß ständig einzelne Muskelfasern sich in *unmerklicher* Kontraktion befinden. Somit also ist der Tonus des Warmblütermuskels, wie experimentelle Untersuchungen von Granit (1955) und *Rudolph (1956)* nachwiesen, weitgehend von den afferenten Impulsen abhängig. So meint Rudolph (1956): „Der Muskel steuert die Erregbarkeit des Motoneurons durch seine sensiblen Impulse selbst. Dieser Mechanismus wird eine umso größere Rolle spielen, je differenzierter die *Arbeitsweise eines Muskels* ist, so etwa bei den Blickbewegungen eines Auges und wahrscheinlich auch bei den differenzierten Bewegungen der Kehlkopfmuskeln, *die im Dienste der* Stimmbildung stehen." Überdies sind sämtliche motorische Funktionen der inneren Kehlkopfmuskeln, aber auch *des* Gaumenbogens und des Rachens, wie Mollnari (1962) experimentell nachgewiesen *hat*, nicht nur von ihren zugehörigen motorischen Nerven, sondern von Nervenästen verschiedener Höhen abhängig, soweit sie ... ben in *einem* tonischen Reflexsystem einbezogen sind. Wir sind daher der Meinung, *daß* die plötzliche Abschaltung einer größeren Anzahl sensibler Impulse, wie es bei der Vagusdurchschneidung der Fall ist, eine reflektorische *Herabsetzung* des Tonus in den jeweils betroffenen Reflexbogen zur Folge haben kann.

Am Gewölbe des weichen Gaumens mit den beiden freihängenden Gaumenbogen und der Uvula, werden *feinste* Abänderungen des Tonus sich

leicht auswirken und sind auch gut zu beobachten. Viel schwieriger aber wird der Nachweis einer Schwächung oder eines Ausfalles der motorischen Aktivität des oberen Kehlkopfnerven gelingen, da dies nur durch Feststellung einer herabgesetzten Spannung der Stimmbänder möglich ist. Diese wird allerdings, wenn auch eine Recurrenslähmung vorhanden ist, symptomatisch als Intermediärstellung des Stimmbandes in Erscheinung treten, wenn keine paralytische Kontraktur den Positionswechsel von paramedian nach intermediär verhindert. Während wir somit bei einer plötzlichen Leitungsunterbrechung des Vagus und seiner Äste die motorischen retrograden Ausfälle durch Funktionsstörung im zugehörigen Kerngebiet, in Folge Ausschaltung größerer sensibler Zuzugsgebiete erklären können, wird bei längerem Bestand einer solchen Lähmung dafür zuzüglich die von Hofer (1944) nachgewiesene retrograde tigrolytische Degeneration der Ganglienzellen im Nucleus ambiguus mitbeteiligt sein.

RESUME

Les observations après section de nerfs et de destructions du nerf vague et de ses rameaux moteurs causées par des tumeurs ont prouvé que non seulement distalement de l'interruption de conduction mais aussi à proximalité de celle-ci, des troubles moteurs peuvent se produire. Après une profonde vagotomie thoracique et abdominale telle qu'elle est effectuée à l'occasion d'une ulcère gastrique on a également pu constater des troubles moteurs rétrogrades du voile du palais.

SUMMARY

Observations carried out after the dissection of nerves and the destruction of the vagus and its branches by tumours, have proved that motorial deficiencies cannot only occur distal to the interruption of motility but also proximal to it. Thus, lesions of the vagus below the branching of the pharyngeal ramus resulted in the paresis of the soft palatum, and flaccid paralysis of the vocal cords in the intermediate position occurred when the nervus recurrens and the nervus vagus were interrupted, while the interruption of the nervus recurrens alone resulted in a paramedian paralysis. Retrograde changing disturbances in motility and in the coordination of soft palatum motions could often be determined after deep thoracic and abdominal vagotomy. It is supposed that the loss of sensory energy in the vagus can reduce the tension of segmentally higher activated muscles, or that in cases of longer interruptions of motility retrograde degenerations of ganglious cells in the nucleus ambiguus can produce similar effects.

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THE INFLUENCE OF HEAT ON THE UPPER RESPIRATORY TRACTS

B GUŠIĆ, Z KRAJINA Z POLJAK, V KONIĆ-CARNELUTTI and I BABIĆ

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The authors examined the upper respiratory tracts of workers in an electric light bulb factory who were exposed to heat Clinical bacteriological and histological examinations were made Parallel examinations were made with albino rats which were exposed to heat during various lengths of time

Heat is transferred from the environment into the body by the physical processes of radiation, conduction, and convection Radiation conduction, convection, and evaporation are the avenues for heat loss from the body About 12% of the body's heat loss is normally dissipated by evaporation of water from the lungs with the warmed exhaled air Excessive heat harms the shocked organism in several ways

1 It produces a widespread superficial vasodilatation and distribution of blood to nonessential parts of the body and compensatory constriction in the splanchnic area

2 Excessive heat increases the metabolism and the oxygen requirement of tissues Overheating the patient necessitates the utilisation of more oxygen and hence leads to a more severe anoxemia, which increases capillary damage and capillary permeability

3 Excessive heat is likely to increase perspiration and consequently promote dehydration.

Increased rates of sweating, decreased concentration of salt in the sweat altered sensitivity of the termoregulatory center and increased blood volume are the various responses with which acclimatisation to heat is explained

Physiological disturbances due to heat stress are evaluated in terms of the body temperature rise, increased cardiovascular demands, diversion of blood to the skin from other organ systems, sweat secretion and water and electrolyte depletion and fatigue In all water-dwelling animals the nasal mucous membrane is the chief mechanism for the loss of heat hence the complexity of the maxillary turbinates which greatly increase the surface area of the nasal mucous membrane The reduction of the ethmoidal max

This w k w s support d by the Council f Sci ntifi Work f Croati

illary and nasal turbinates in man and the higher primates is probably a factor concerned in the transformation of the head and the relation between respiration and olfaction The degree of heat loss taking place through the nasal mucous membrane depends upon the vascularity moisture and the area of the mucous membrane In man the area of the nasal mucosa is reduced as the skin becomes a more efficient mechanism for the regulation of heat loss, but there is nevertheless a complex functional correlation between the nasal mucous membrane and skin which is controlled by the autonomic nervous system and certain ductless glands. In this way we can explain the noxious effect of heat on the upper respiratory tracts by a twofold mechanism The effect of heat itself on the mucous membrane and the reflectory mechanism of the skin—the mucous membrane of the nose.

Our Investigations

Our investigations regarding the influence of heat on the respiratory mucous membrane were made experimentally on rats and by clinical examination of the workers of an electric light bulb factory who working 8 hours a day were exposed to temperatures from 30 to 51.5 C. As we had no experience of the behaviour of rats at high temperatures (and could find no references in the literature) we chose different temperatures, beginning with 40 C during various l ngths of time Rats having no sweat glands, their general reaction is much more pronounced, a fact we had to take into consideration. These investigations consisted of histological studies of the nose and trachea.

(a) The findings in the nose and pharynx

The mucous membrane of the nose was dry glossy and pink. In the nasal vestibule there was dried up secretion In *eight cases* there were initial atrophic changes of the posterior part of the *nose bilaterally* in three cases unilaterally In one case there was a typical picture of ozaena In 37 cases the mucosa of the pharynx was dry with accumulated desiccated secretion and granular formations.

(b) The diseas of the espiratory tra t in the course of duration of work

Frequent colds	27 cases	Bronchitis	3 cases
Ang na	20 cases	P eumonia	2 cases
Sore throat	8 cases	Bleeding of the nose	2 cases
Sinusit	3 cases		

(c) Othe diseases in th course of the duration of work

Headaches	17 cases
Psychoneurosis	10 cases

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TABLE 1 *Experimental animals (31 albino rats) Histological findings of the mucosae of the nose and the trachea*

Group I (Figs. 1 d 2) Three days of 7 hours exposure t 40°C. Sev lmal

Nose Minor hanges of the perficial epithelium, strong glandul hyperpl ia nd increased i traepith lial gl ds, diffuse i filtration, poradic bleedi g

Trachea. Small i filtrates in pars membranace three times, ormal findi gs f times

Group II (Figs. 3 and 4) T enty days of 3 hours exposure t 30°C. Fi animal

Nose Changed epithelium with medi m i filtrates i the mucosa twice, bleeding in the mucosa with stronger glandular hyperplasi twice, ormal findings once

Trachea. Minor infiltrates i the mucosa with enlarged gland four times. St g inflammation of the mucosa once

Group III (Figs. 5 nd 6) First week 3 hours exposure per day at 30 C. Second week 3 hours exposure per day t 40°C. Third week 4 hours exposure per day at 40°C. Fourth eek 4 hours exposure per day at 40°C. Eight nimals

Nose Changed epithelium, glands hanged nd disintergrated, enl rged blood essel with bleedi g nd inflammatory inf ltrates

Trachea. Cha ged epithelium in lesser degree with mucou surface subepithelially en larged blood essels, oedema, sporadic bleeding, nd maller i fl mmatory infiltrates

Group IV (Figs. 7 nd 8) Thirty-one days of 7 hours exposure t 33 C. Seven imals

Nose The cha ges of the epitheli m, gland hanged, in some places degenerated, bleeding in the mucosa nd pronounced i flammatory infiltrates

Trachea. Cha ged epitheli m i lesser degree, i one case papill ry hyperplasia of the epithelium, infiltrates in pars membranacea, poradic bleeding

Group V (Figs. 9 nd 10) Fifteen days f 7 hours exposure t 40°C. Fifteen days i normal conditions. Fou animals

Nose Epithelium regular glands very merou in the stage of recovery asodilat tion infiltration medi m

Trachea. Slight infiltrates i pars membranacea with enlarged gland

—

(f) Bacteriological findings

I

Potentially pathogenic flora	*Potentially pathogenic flor and saprophytic flora*	*Saprophytic flora*	*Sterile*
23	9	44	5

II

Potentially pathogenic flora							*Saprophytic flora*					
STA	P	HI	PR	E	STH	C	STA	SN	SC	STV	STAH	D

Ad oto-laryng 6



(*d*) *X ray of the sinuses (46) workers*

Negative fi ding	6 case
Positi finding	40 case

(*e*) *Smear of the nose*

M cu	2 ases
Epithelial cells	28 cases
Leucocytes	50 ases
Eosin phyles	15 cases
Microorg nisms	16 cases

TABLE 2

Examined workers of the electric light bulb factory exposed to the following conditions:	
1. The air temperature of working premises	27–45 C
2. Globus temperatur	33–45.5 C
3. Relative humidity	37–55%
4. The velocity of air streaming, 87 workers	Up to 0.01 m/sec
Women workers: 71	Age 19–48
Men workers: 16	Duration of work 1–18 yrs

TABLE 3

Bacteriological control group of health students (120)

[illegible] swabs

Sterile	54 cases
Pathogenic	6 cases
Saprophytic	52 cases
Saprophytic + pathogenic	8 cases

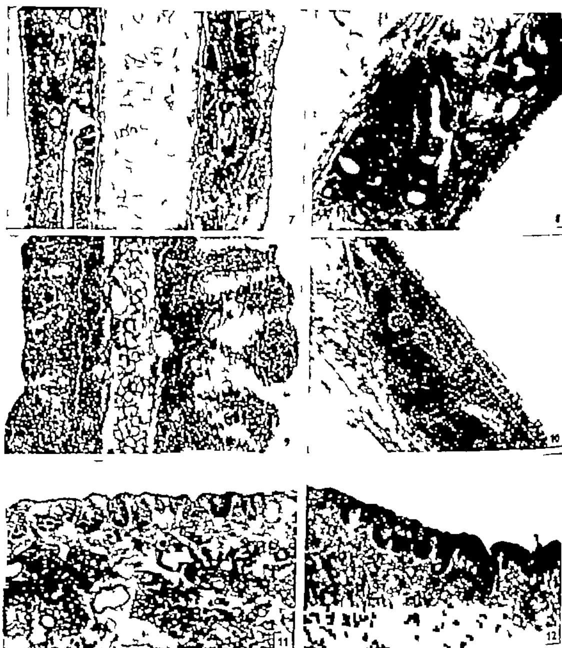
(*g*) *Pathohistological findings of the nasal mucosa in 12 cases*

There was an alteration of the superficial epithelium leading to a squamous form, an enlarged basal membrane vasodilatation and oedema and, in one case, fibrous changes, numerous glands, and microcellular infiltration (three cases)

(*h*) *Electrophoresis of proteins was made in 30 cases*

Twenty cases were exposed to a temperature above 35 C and ten cases were exposed to a temperature up to 35 C. In all cases the values of the proteins and gammaglobulins were normal.

We have found histologically that the nasal mucosa reacts to heat as well as other external noxae, and ranges within the frame of the reaction of its compound elements. The change of the epithelium up to the creation of a squamous form, which, in the normal condition of the nasal mucous membrane returns again to its cylindrical form. Hyperplasia of mucous glands and the increase of goblet cells on the surface is a satisfactory reason for the increased necessity to produce mucous. The glands did not decrease numerically even at higher temperatures, but disintegration processes took place which led to the disfunction and degeneration of glands. Oedema and vasodilatation are always *present*, *while fibrosis* was less frequently met with (as with human beings) we suppose that vasodilatation by heat is a more constant form which prevents the formation of fibrous tissue. In cases where the rats were given normal conditions, as early in the course of a



Explanat on

STA + – Staphylococcus pyogenes ureus coagulase +
P – Pneumococcus
HI – H mophylu infl enzae
PR – Proteu
E – Enterococcus
STH – Streptococcus haemoliti us
C – Candida

STA – – Staphylococcus aureus and lbus coagulase –
SN – Saprophytic N isseriae
SC – Saprophytic undifferentiated cocci
STV – Streptococcus viridans
STAH – Streptococcus nhaemolitycus
D – Diphteroid

ZUSAMMENFASSUNG

Die Autoren haben die oberen Atmungswege bei Arbeitern einer Fabrik elek t ischer Glühbirnen die der Hitze ausgesetzt werden, untersucht. Es wurden klinische bakteriologisch und histologische Untersuchungen unternommen. Parallel wurden ebenfalls Untersuchungen bei Albino-Ratten, die einer Wärme von verschiede er Dauer usgesetzt waren vorgenommen

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fortnight the nasal mucous membrane returned to histological normalisation The changes of trachea were less frequent and to a much smaller degree as we could see with other physical damage to the nasal mucous membrane.

Our examinations of workers also gave interesting data. Anamnestically the workers complained of the frequent appearance of the common cold, *sore throat, and angina. Not in a single case did* we find a normal mucosa of the nose during the work. The changes ranged from dryness, the injection of the mucosa itself and, less frequently the beginning of athrophic changes. Only in one case did we find a typical picture of ozaena. These clinical findings correspond to histological analysis of the nasal mucosa of the workers and experimental animals Bacteriologically we found an increased positive finding of bacterial flora as well as a large percentage of leucocytes in the smear of the nose These findings prove the susceptibility of the nasal mucosa after the influence of heat upon the bacterial and virus infection, and the possibility of the appearance of colds, angina, and pharyngitis The changes in the X ray pictures of the sinuses are very frequent, but only a few workers were treated for sinusitis We suppose that the mucosa of the paranasal sinuses reacts to the noxa which influences the nasal mucosa, with stronger oedema because of the looser structure of the mucosa of the sinuses. Among general anamnestical data, we found that the workers suffer from headaches and in 10 cases we could clinically establish psychoneurosis We cannot go into the explanation of the relation between the headaches and damage to the nasal mucosa, but in other cases of dryness of the nasal mucosa we came across appearances of neuralgia and headache Very probably the reflexes play a certain role here

Eosinophyls in the smear of the nose in as many as 15 cases is probably connected with the greater liberation of histamine in the mucosa damaged by heat

Accordingly heat influences the mucosa of the nose by producing disturbances of the normal physiological protective function of the nasal mucosa with repercussions upon the paranasal sinuses and pharynx The principal mechanisms of this disturbance are the changes of the epithelium and nasal mucus, lysozimes as the protectives factors, and oedema of the tissue which slows down the biochemical processes However here too broad possibilities of regeneratory and reparatory abilities of the nasal mucosa come into consideration

RESUMÉ

Les auteurs ont examiné les voies respiratoires supérieures chez les ouvriers d une usine d ampoules électriques. On a procédé aux examens cliniques, bactériologiques, histologiques. Paralellement on a examiné de la même manière les rats albinos qui ont été exposés à la chaleur d une durée variable

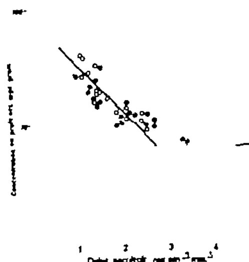


FIG. 1 Relation entre la concentration en protéines et le débit sécrétoire chez les sujets normaux. ● Prélèvements réalisés en novembre; O prélèvement réalisés en juillet et août.

MATÉRIEL ET MÉTHODES

Le mucus a été prélevé chez deux groupes de sujets.

Le premier groupe est constitué de 9 patients affectés d'une sensibilisation nasale purement périodique aux pollens de graminées. Ces patients présentent chaque année entre mai et juillet, les manifestations cliniques du rhume des foins.

Le second groupe comprend 10 patients dont les crises sont déclenchées exclusivement par des facteurs d'irritation locale d'ordre physique ou chimique. Ces patients qui selon l'expression de Melchior (1950) souffrent d'éréthisme nasal d'origine exogène ne présentent aucun signe anamnestique clinique ou biologique de sensibilisation allergique.

Le prélèvement du mucus a été pratiqué, comme chez les *sujets normaux*, à l'aide de fragments rectangulaires de papier filtre placés au contact de la

TABLEAU 1 *La composition inorganique du mucus nasal*

Dosages réalisés chez 40 sujets normaux.

	Valeurs extrêmes (µeq·g⁻¹)	Valeurs moyennes (µeq g⁻¹)
Concentration en Na	120,09 et 135,92	127,70
Concentration en Cl	128,83 et 148,99	138,83
Concentration en K	12,37 et 25,01	17,30
Concentration en Ca	3,23 et 6,77	4,88

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ÉTUDE COMPARATIVE DE LA COMPOSITION CHIMIQUE DU MUCUS NASAL PRÉLEVÉ AU COURS DES HYPERSÉCRÉTIONS D'ORIGINE RÉFLEXE ET ALLERGIQUE

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Deux composants du mucus subissent des variations de concentration différentes suivant le type d'hypersécrétion Il s'agit du K et des glycoprotéines. Au cours des hypersécrétions d'origine réflexe, la concentration en K augmente et la concentration en glycoprotéines diminue tandis qu'au cours des hypersécrétions d'origine allergique la concentration en K diminue et la concentration en glycoprotéines augmente. Le mécanisme de ces modifications est discuté ainsi que leur intérêt diagnostique

Le mucus nasal prélevé chez des sujets normaux, dans des conditions techniques rigoureuses, présente une composition chimique bien définie Nous avons montré dans un travail précédent, que les concentrations moyennes des éléments inorganiques, évaluées en microéquivalents par gramme, sont de 127 7 pour le Na, de 138 8 pour le Cl de 17 3 pour le K, de 4 8 pour le Ca. Les concentrations limites sont indiquées dans le Tableau I elles sont constantes, quel que soit le débit sécrétoire mesuré (Mélon & Schoffeniels, 1966)

Il n'en est pas de même pour la concentration en glycoprotéines qui varie fortement avec la sécrétion pour des débits inférieurs à 3 mg cm^{-2} min^{-1} Il existe une relation linéaire inverse entre les deux paramètres autrement dit, plus le débit augmente, plus la concentration en glycoprotéines diminue (Mélon *et al* 1968) La concentration en glycoprotéines peut être évaluée aussi bien par le dosage de la fraction mucopolysaccharidique que par le dosage de la fraction protéinique Celle-ci mesurée par la réaction au biuret, présente une concentration descendant de 40 à 5 mg g^{-1} pour des débits échelonnés entre 1 et 3 mg cm^{-2} min^{-1} Au-delà de ce dernier débit la concentration en protéines dont la valeur moyenne est de 6 5 mg g^{-1} ne se modifie plus (Fig 1)

Le présent travail a pour but de présenter les résultats d'une étude comparative de la composition de la sécrétion nasale prélevée chez des sujets atteints de coryza spasmodique d'origine allergique ou d'origine réflexe

Nous avons recherché s'il existait des critères d'ordre chimique permettant de distinguer l'un de l'autre, les deux types de coryza.

TABLEAU 3 *Evolution du débit sécrétoire et de la composition chimique du mucus nasal au cours de la crise allergique (Patient n 7)*

Sensibilisation au pollen de graminées.

Prélèvements	Débit sécrétoire (mg cm^{-2} min^{-1})	Na (µeq./g)	K (µeq./g)	Ca (µeq./g)	Cl (µeq./g)	Protéines (mg.g^{-1})
I. 5 min. après pulvéris. sol. saline témoin	2,09	131,51	12,91	8,66	145,30	23,37
II 8 min après pulvéris. sol. antigénique	16,38	110,99	9,80	3,49	128,54	17,47
III 15 min. après pulvéris. sol. antigénique	21,41	115,23	9,02	2,33	115,32	18,03
IV 30 min. après pulvéris. sol. antigénique	17,28	116,42	10,95	3,01	118,85	7,59
V 40 min. après pulvéris. sol. antigénique	17,71	109,48	9,10	3,24	116,03	11,00
VI 60 min. après pulvéris. sol. antigénique	14,39	113,34	11,67	2,49	110,45	5,30

niveau avant le contact antigénique Lorsque la concentration initiale est inférieure à 12 mg.g^{-1} elle présente une brusque augmentation dès le début de la crise et atteint une valeur 2 à 5 fois supérieure à celle de départ Cette concentration élevée en protéines ne se maintient que pendant 10 à 20 minutes Elle diminue dans la majorité des cas et rejoint vers la 60me minute, le niveau mesuré chez les sujets normaux si l'on tient compte du débit sécrétoire à ce moment (Tableau 2)

Lorsque la concentration initiale est supérieure à 12 mg.g^{-1} elle subit une diminution mais reste néanmoins toujours supérieure aux valeurs normales pendant la plus grande partie de la crise (Tableau 3)

Comme chez les sujets normaux, la fraction mucopolysaccharidique suit une évolution strictement parallèle à la fraction protéinique, ce qui signifie par conséquent que c est la concentration en glycoprotéines qui a évolué pendant la crise.

B. Au cours de la crise de coryza d'origine réflexe les concentrations en Na, Ca et Cl subissent par rapport aux valeurs normales, une diminution d'autant plus importante que l'hypersécrétion est plus abondante. La valeur moyenne de ces différentes concentrations est de 10 µeq.g^{-1} pour le Na, de 4 28 µeq.g^{-1} pour le Ca, de 122,83 µeq.g pour le Cl

La concentration en K ne varie pas de façon significative La valeur moyenne est de 1 19 µeq.g^{-1}

La concentration en protéines est comparable aux valeurs déterminées pour les sujet sains dont le débit sécrétoir dépasse 3 mg.cm^{-2} min^{-1} (Tableau 4)

TABLEAU 2 *Evolution du débit sécrétoire et de la composition chimique du mucus nasal au cours de la crise allergique (Patient n 5)*

Sensibilisation au pollen de graminées.

Prélèvements	Débit sécrétoire (mg.cm ² min)	Na (µeq /g)	K (µeq /g)	Ca (µeq./g)	Cl (µeq /g)	Protéines (mg g⁻¹)
I Sur muqueuse saine	2,12	131 43	15,92	5,61	147,68	11,80
II 5 min. après pulvéris. sol. saline témoin	2,23	133,18	16 05	5,40	145,21	11 75
III. 5 min après pulvéris. sol. antigénique	33,39	118 00	10 43	4 03	123,14	23,84
IV 10 min. après pulvéris. sol. antigénique	30 82	118,42	12,48	4 13	116,18	18,37
V 25 min. après pulvéris. sol. antigénique	20,54	119,32	11,23	4 73	122,06	15,00
VI 60 min après pulvéris. sol. antigénique	17,81	118,88	12,64	4,58	124,63	8,71

muqueuse septale Pendant la période d'imbibition des papiers collecteurs, le vestibule nasal est obturé à l aide d un pince-nez. Le mucus récolté est pesé puis dilué en vue du dosage des éléments inorganiques Na, K, Ca et Cl, ainsi que des protéines.

Chez les sujets du groupe I les prélèvements de sécrétion sont réalisés d abord au niveau de la muqueuse septale saine, ensuite cinq minutes après la pulvérisation d une solution témoin ne contenant pas de pollen, enfin, à plusieurs reprises après l application de l allergène Cette application est réalisée en dehors de la période de pollinisation.

Chez les sujets du groupe II la crise est déclenchée par le simple contact du papier collecteur

RESULTATS

A Ni le contact du papier collecteur ni la pulvérisation nasale de la solution témoin ne déclenchent chez les sujets du groupe I d'hypersécrétion ou de modification quelconque de la composition chimique du mucus.

Par contre, la pulvérisation locale de la solution antigénique spécifique provoque l apparition d une violente crise de coryza spasmodique. Au cours de celle-ci, les concentrations en Na, K, Ca et Cl diminuent La diminution moyenne est évaluée à 13 5 % pour le Na, 35 % pour le K, 43 % pour le Ca et 20 % pour le Cl Cette diminution des concentrations en éléments inorganiques se maintient pendant toute la durée de la crise (Tableau 2)

La concentration en protéines subit une évolution différente suivant son

(*a*) La concentration en K est très variable d'un sujet normal à l'autre. Mesurée chez 40 sujets sains, elle oscille entre 12 et 25 µeq.g^{-1}. Si l'on ne connaît pas le niveau de cette concentration immédiatement avant la crise d'hypersécrétion, on ne pourra considérer comme valeurs anormalement basses et donc typiques d'une étiologie allergique que les concentrations inférieures à 12 µeq.g^{-1} et comme valeurs élevées typiques d'une étiologie réflexe que les concentrations supérieures à 25 µeq.g^{-1} dont il faut déduire l'abaissemment, évalué à 35% que provoque la réaction anaphylactique — soit 17 µeq.g^{-1}. Ainsi, il apparaît que la mesure de la seule concentration en K ne permettra de déceler l'étiologie que d'un nombre limité d'hypersécrétions paroxystiques.

La mesure de la concentration en protéines est un élément de diagnostic plus sûr. En effet, des concentrations supérieures à 5 ou 7 mg.g^{-1} mesurées au cours des minutes qui suivent le début de la crise doivent être considérées comme témoignant d'une réaction allergique pour autant que l'existence d'un infection locale intercurrente puisse être éliminée.

(*b*) Le prélèvment de la sécrétion chez un sujet dont le débit sécrétoire est normal ne peut s'effectuer qu'à l'aide d'un matériel hydrophile placé au contact de la muqueuse. Il s'avère donc impossible de connaître la composition du mucus avant la crise, chez des sujets atteints d'hypersécrétion d'origine réflexe puisque le prélèvement déclenche la paroxysme sécrétoire. Il en a de même pour la majorité des allergies nasales chroniques, au cours desquelles se développe progressivement une hypersensibilité tactile de la muqueuse.

D'autre part, au cours des manifestations sécrétoires de ces allergies chroniques, le prélèvement du mucus devra se faire sans attouchement de la muqueuse si l'on veut éviter la production simultanée d'une hypersécrétion réflexe venant modifier la composition chimique du produit de l'hypersécrétion d'origine anaphylactique. Comme nous l'avons montré plus haut, les modification d'origine réflexe de la composition de la sécrétion consistent notamment en une augmentation de la concentration en K et en une diminution de la concentration en protéines. Aussi, l'irritation tactile de la muqueuse nasale au cours d'une crise d'origine allergique risquera-t-elle de faire perdre à la concentration en K toute valeur diagnostique. Par contre, il persistera toujours une élévation de la concentration en protéines témoignant de la réaction anaphylactique.

CONCLUSIONS

Il existe des différences entre la composition chimique de la sécrétion nasale d'origine allergique et celle de la sécrétion d'origine réflexe : elles concernent principalement les concentrations en K et en protéines. Au cours des manifestations sécrétoires d'origine anaphylactique, la concentration en K diminue tandis que celle en protéines augmente. Au cours des hypersécrétions d'origine réflexe, la concentration en K reste élevée comme chez

TABLEAU 4 *Composition du mucus nasal récolté au cours de la crise de coryza spasmodique d'origine réflexe*

Sujets	Age et Sexe	Débit sécrétoire (mg cm⁻² min)	Na (µeq /g)	K (µeq /g)	Ca (µeq /g)	Cl (µeq /g)	Protéines (mg g⁻¹)
1 Pir A	40 a. F	11 67	114,58	17 77	3,33	129,75	6,41
2. Koc M. L.	36 a. F	13 14	101,58	17 60	4 46	118,82	[illegible]
3 Hex. P	2[illegible] a F	15,87	116,89	15,81	6,81	132,32	4,39
4 Rig. L.	21 a. F	1 [illegible] 0	104,36	[illegible]	6,30	128,10	5,12
5. Pir J	6 a F	18 00	11 [illegible] 46	16 28	3,65	130,64	7,31
6. Méd C.	23 a F	18,33	113,57	18,56	4,83	121,80	6,21
7 Dem J P	27 a M	18 81	109 12	15,10	2,90	120,36	7,22
8 Con J	34 a. F	19,20	95 78	1 [illegible] 75	2,68	108,99	5,39
9 Ams. P	[illegible]1 a. F	24 00	93,83	16,[illegible]5	4 83	121,32	5,66
10 Cho. J	20 a. F	25 [illegible]0	104 02	16,14	3,30	114,25	6,70
Valeurs moyennes		18,31	107 15	17 19	4,29	122,63	6,02
Valeurs moyennes normales		1 à 3	127 [illegible] 0	1 [illegible],30	4,88	135,82	5 (pour les débits > à 3 mg)

DISCUSSION

Que l origine de l hypersécrétion soit allergique ou réflexe, les concentrations en Na, Ca et Cl évoluent dans le même sens toutes trois diminuent Les concentrations en Na paraissent plus basses dans les cas d hypersécrétion d origine réflexe tandis que les concentrations en Ca et en Cl semblent diminuer davantage dans les cas d hypersécrétion de nature anaphylactique Mais nos recherches n ont pas porté sur un nombre suffisant de patients pour que des limites de concentrations correspondant à l un ou l autre type de coryza puissent être établies

Par contre deux composants du mucus — le K et la fraction protéinique — présentent une évolution divergente suivant que l'hypersécrétion est d origine allergique ou réflexe Dans la première éventualité la concentration en K diminue, celle en protéines augmente dans la seconde éventualité, la concentration en K garde une valeur très élevée comparable à celle mesurée chez le sujet normal tandis que la concentration en protéines présente des valeurs très basses

Sur le plan pratique, les valeurs de concentration en K et en protéines mesurées chez un patient atteint d hypersécrétion paroxystique peuvent-elles être utilisées en vue d un diagnostic étiologique? Pour répondre à cette question, il faut prendre en considération, d une part, les niveaux significatifs de concentration des deux composants du mucus et, d autre part la méthode de prélèvement de la sécrétion

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PERMEABILITY OF THE INNER EAR MEMBRANES

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Soluted substances can penetrate through epithelial layers either by free diffusion through the intercellular spaces or by transcellular transport. By injection of *th.* as a tracer into peri- and endolymph we found a typical transcellular transport of the tracer through Reissner's membrane in micropinocytosis vesicles. Contrary to this observation, the tracer entered the "cortilymph spaces" from sc. tympani by free diffusion through open intercellular spaces. The sensory cells take up the tracer at their infranuclear area. We therefore conclude that cortilymph is perilymph and the hair cells of Corti's organ are mainly supplied from the perilymph side.

We know two ways in which soluted substances can penetrate an epithelial layer: either through the cells or between their intercellular spaces. Since the investigations of Farquhar & Palade (1965) we assume that there is no free passage through intercellular spaces closed by a "Zonula occludens". Apart from other mechanisms without morphological characteristics, soluted substances can be transported through epithelial cells by membrane vesiculation. This mechanism we call "pinocytosis" or rather "cytopempsis" (Moore & Ruska, 1957). Several authors have described similar observations at different parts of the cochlea.

To study further details of ion and fluid transport between the endolymphatic and perilymphatic spaces we injected a colloidal solution of thorium dioxide (*th*) as a tracer into the endo- and perilymph of the guinea pig. Because of their small size (40 Å) these electrondense particles are suitable as a tracer for cochlear study by electronmicroscopy. We present our findings at Reissner's membrane and the organ of Corti using this method.

Five minutes after injection of the tracer *into the* perilymph we found that the intercellular spaces (ICS) between the connective cells of Reissner's membrane on the scala vestibuli side *were filled with th*, as well as the basement membrane. Furthermore, *th penetrated* into the ICS between the epithelial cells, passed through the desmosomes, and was finally stopped by the Zonula occludens. We never saw the tracer *within the Zonula* occludens or desmosomes themselves. *At the* basal and junctional surfaces of the epithelial cells we found micropinocytosis vesicles (MPV) crossing their cytoplasma to the *endolymphatic surface* within *30-40* min. Sometimes we observed MPV *pouring out their contents into the endolymph.*

le sujet normal tandis que celle en protéines est très basse. Ces différences peuvent servir de critères diagnostiques permettant de distinguer les deux types d hypersécrétion Ne sont cependant significatives que les concentrations en K inférieures à 12 μeq g^{-1} ou supérieures à 17 μeq.g^{-1} mesurées dans des échantillons de mucus prélevés en l absence de toute irritation de la muqueuse Par contre en l absence d infection locale, toute élévation de la *concentration en protéines au-dessus* d une valeur de 5 à 7 mg.g^{-1} témoigne d une réaction allergique qu il y ait ou non des manifestations réflexes concomitantes.

ZUSAMMENFASSUNG

Es bestehen Unterschiede zwischen der chemischen Zusammensetzung der Nasensekrete allergischen Ursprunges und der Sekrete reflektorischen Ursprunges dieses betrifft hauptsächlich die Konzentration an K und an Proteinen. Im *Laufe der Absonderungsvorgänge (= Sekretion) anaphylaktischen Ursprunges* wird die Konzentration an K kleiner während diejenige an Proteinen größer wird.

Im Laufe der übergroßen Absonderung (= Hypersekretion) reflektorischen Ursprunges bleibt die Konzentration an K sehr hoch so wie beim normalen Menschen dagegen die an Proteinen sehr klein

Diese Unterschiede können als Diagnose Erkennungszeichen dazu dienen, die zwei Arten Hypersekretionen zu unterscheiden Doch die Konzentrationen an K sind nur von Wichtigkeit wenn sie tiefer liegen als 12 ueq.g^{-} oder höher als 17 ueq.g^{-} und wenn die Schleimproben in Abwesenheit jeglicher Entzündung der Schleimhaut gemacht wurden In Abwesenheit jeglicher örtlichen Entzündung zeigt dagegen jede Erhöhung der Konzentration an Proteinen über 5 bis 7 mg.g^{-} eine allergische Reaktion an, ganz gleich ob begleitende reflektorische Äußerungen bestehen oder nicht

SUMMARY

There are differences between the chemical composition of nasal secretion of allergic origin and that of reflex origin they mainly concern K and protein concentrations. During *secretory manifestations of* anaphylactic origin K concentration decreases, while that of protein increases. In hypersecretions of reflex origin, K concentration remains high as in normal individuals, while that of proteins is very low These differences may serve as diagnostic criteria for distinguishing both types of hypersecretion However only those K concentrations lower than 12 μeq.g^{-} or higher than 17 μeq.g^{-} measured on mucous samples taken from mucosa devoid of any irritation have any significance On the contrary when there is no local infection any rise in protein concentration higher than 5 to 7 mg.g^{-} evidences an allergic reaction whether or not there are any concomitant reflex manifestations.

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ever there is no free communication between endo- and perilymph through the ICS of the epithelial cells as they are sealed by impermeable terminal bars. Thus an active transport through the epithelial cells by means of pinocytosis and/or cytopempsis is involved.

In the organ of Corti and its supporting structures we have a completely different situation. Between perilymph on one side and endolymph on the ther there is a third fluid space which was called the cortilymph space" by Engström (1960) Several electrophysiological and biochemical investigations suggested that cortilymph and perilymph should have the same composition However no connection has been demonstrated so far between the two fluids by electronmicroscopy After the injection of *th* into the endolymph we saw the tightly packed particles on the endolymphatic surface of the sensory cells and the reticular membrane The intervening intercellular spaces, sealed by marked Zonula occludens, could not be passed by the *th* particles. There was no uptake of *th* to be seen in this region. Contrary to this finding, the tracer injected into the scala tympani passed fter only a few minutes through the tympanic covering-layer and the fibers of the basilar membrane. Thirty to sixty minutes later the particles reached the cortilymph spaces through the ICS. We saw the tracer particles between the Deiters cells surrounding the outer spiral fibers between the pillar cell and the unmyelinated nerve fibers at the habenula perforata, so that the particles were lining the surface of the cortilymph spaces. Further the labyrinth of the ICS around and below the inner hair cells contained *th* particles ad ancing up to the terminal bars at their hair bearing surface

Even the synaptic cleft between the inner and outer hair cells and their nerve endings contained *th* particles. We demonstrated an uptake of the *tracer into the sensory cells at their infranuclear surface* (Fig 1)

With our method we have shown that the sc tympani and the tunnel spaces communicate freely through open ICS but are closed against the subtect rial endolymph. We therefore have proved that the so-called "cortilymph spaces" contain perilymph. This means that the outer and inner hair cells of the organ of Corti will be mainly if not completely supplied *with nutritious substances from the perilymph.* We assume that the oxygen upply f the sensory cells occurs *from the perilymphatic side too.*

Ref rring to our diagram, shown at the beginning, of two different parts of the cochlea we could demonstrate that both transcellular transport as well a intercellular diffusion play an important role in the inner ear fluid balance

RESUME

La inject t d thorium dioxid d ns la peri- et d n l'end lymphe nous a ons xaminé les structures membraneuses de l'oreille intern pa rapport à leurs caractéristiques d perméabilité et d résorption La scala medi fonctionnait comme



FIG. 1 Infranuclear regi n of an inn hai cell 60 min ft i jectio of thorium di xid int th perilymph Th trace can be seen in th ynapti left (*SyC*) between the i n hai cell (*IHC*) nd a esicul ted nerve endi g (*NE*) M y micropinocytosis vesicles tak up th tracer into th hai cell and even into the n rve cell (*arrows*)

After injection into the scala media the same mechanism in the opposite direction was to be seen *th* could not pass through the Zonula occludens of the ICS from this side either Numerous tracer filled MPV invaginated into the epithelial cells from the endolymphatic surface, travelling through their cytoplasma towards the perilymph We often saw MPV-containing *th* entering the typical cysternae of Reissner s membrane thus forming a sort of "multivesiculated body"

By this method Reissner s membrane was proved to be permeable for fluid transport from either side. The layer of connective like cells and the basement membrane allowed a free passage even for macromolecules. How

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STREPTOMYCINSPIEGEL IN DER PERILYMPHE DES MENSCHEN

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In früheren Untersuchungen wurde durch Stupp im Tierversuch am Meerschweinchen gezeigt, daß die Aminoglykosidantibiotica (Streptomycin, Kanamycin) in der Peri- und Endolymphe sehr hohe Konzentrationen und sehr lange Verweildauer erzielen. Der gleiche Effekt wird nunmehr auch in der Perilymphe des Menschen für parenteral injiziertes Streptomycin in therapeutischer Dosierung nachgewiesen.

Das Schrifttum über die ototoxischen Wirkungen der Aminoglykosidantibiotica ist fast unübersehbar geworden. Verhältnismäßig wenige Untersuchungen beschäftigen sich jedoch mit den pharmakokinetischen Aspekten dieser Frage. Wir sind der Überzeugung, daß dieser Aspekt nicht nur mit den bereits vorliegenden tierexperimentellen Befunden sehr interessante Ergebnisse in der speziellen Frage der Ototoxikose erbracht hat, sondern darüber hinaus geeignet ist, zu allgemeinen physikochemischen Fragen der Innenohrbiologie einen Beitrag zu leisten.

Pharmakologische Wirkungen und toxische Nebenwirkungen *können nur* zustande kommen, wenn das Pharmakon in ausreichender Menge bzw. Konzentration mit den reagierenden Bestandteilen der *Zelle* in Kontakt kommt. Blut- bzw. Plasmaspiegel einer Substanz sind keineswegs ohne *weiteres für* die Konzentration der Substanz am Wirkungsort repräsentativ. Für die sehr unterschiedliche Verteilung der Substanzen im Organismus und für ihren Verbleib sind Resorption, Distribution und Elimination ent*scheidend*.

Für derartige pharmakokinetische Untersuchungen an der Innenohrlymphe sind Natrium- und Kaliumionen, Glukose usw. *nicht so sehr geeignet*, wie es auf den ersten Blick erscheinen mag. Die Vielseitigkeit ihres ubiquitären Vorkommens, ihrer Stoffwechselaktivität und ihres Transportes im Organismus macht die Beurteilung sehr schwierig.

F[illegible]ind daher Substanzen, die sich durch definiertere und überschaubare Eigenschaften auszeichnen, geeigneter, wie z. B. Inulin, *das bei Clearance*-Untersuchungen der Niere Anwendung findet. Das chemisch indifferente und nicht resorbierbare Inulin, das nicht in die *Zelle* eindringen und infolgedessen auch nicht sezerniert werden kann, kann nur durch Filtration oder Diffusion transportiert werden. *Inulin gelangt nach Untersuchungen* von Stupp zwar ohne Schwierigkeiten in die Perilymphe, *aber sehr schwer* wieder heraus. Die verzögerte Ausscheidung ist *eine Folge unzureichender* Rückresorption. Diese „Inulinclearance" des Innenohres ergibt nun *later*

An Hr *Wersäll* Einen toxischen Effekt des Th. sahen wir bei unseren Versuchen noch nicht Es stellt sich möglicherweise nach längeren Inkubationszeiten erst ein. Die Pinocytose des Th. aus dem synapt. Spalt konnten wir nach 60 min bis etwa in kernähe der Sinneszellen (Golgi Zonen?) verfolgen An der Stria vasc. findet eine deutliche Resorption des Th. aus der Endolymphe statt.

An Hr *Meyer zum Gottesberge* Eine freie Diffusion durch Reissner Membran halten wir aufgrund unserer Beobachtungen für sehr unwahrscheinlich. Möglicherweise wird Inulin transzellulär transportiert.

An Hr *Engström* Nachdem Makromoleküle bis 40 Å Grösse von der sc. tympani in die „Corti lymphräume gelangen können ist kaum anzunehmen, dass ein Unterschied zwischen Inhalt der Cortilymphräume und der Perilymphe besteht.

To Mr *Palva* By our method we got no information on any fluid movement, neither perilymph nor endolymph.

To Mr *Spoendlin* Yes, we sometimes see terminal bars at the basilar membrane But they seem not to close the whole length of the ics, so that the tracer can pass them We too are irritated by the very few particles in the tunnel space. But they penetrate very slowly After a long period there might be more of them However small amounts of additional active transport into this region cannot be excluded. Limbus spiralis apart from epithelial cells, the whole space is filled with perilymph. Interdental cells take up great amounts of the Th. by pinocytosis (slide)

à Mr *Portmann* En effet le scala media est un espace totalement fermé par son epithelium et il n y a pas d échange entre péri et endolymph par diffusion. Par notre méthode il est impossible de dire quelque chose sur le movement de l'endolymphe et l endroit de sa production.

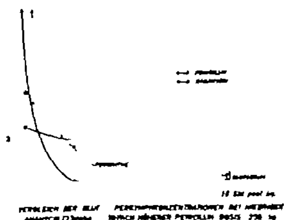


Abb. 2. Vergleich der Blut- und Perilymphkonzentrationen bei niedriger Kanamycin- (25 mg/kg) und 10fach höherer Penicillindosis (250 mg/kg)

Polymyxine, welche neurotoxisch, aber nicht ototoxisch sind, gehen gleichfalls nicht in die Innenohrlymphe über.

Zur Behandlung entzündlicher Innenohrerkrankungen sollten daher nur solche Antibiotica herangezogen werden, die am Wirkungsort eine hohe Konzentration erreichen. Das sind die ototoxischen Antibiotica. Jedoch muß bei entzündlichen Innenohrerkrankungen die Dosierung der ototoxischen Antibiotica niedrig gehalten werden, da nach den Untersuchungen von Voldrich & Vabreck unter entzündlichen Bedingungen im Innenohr besonders hohe Konzentrationen dieser Antibiotica auftreten.

Von theoretischem und klinischem Interesse ist nun die Frage, ob sich die erwähnten, im Tierexperiment am Meerschweinchen erhobenen Befunde auch beim Menschen bestätigen lassen. Ergänzend zu den tierexperimentellen Befunden wurden daher analoge Untersuchungen am Menschen durchgeführt, die uns die Frage beantworten sollten, ob beim Menschen ebenfalls eine spezifische Kumulation der toxischen Antibiotica vorkommt, die für die Ototoxikosen ursächlich verantwortlich gemacht werden kann.

Die Untersuchungen wurden an insges. 26 Otosklerosepatienten vorgenommen, bei denen die Gelegenheit gegeben war, anläßlich einer hörver-

Stunde post inj.	Endolymphe	Perilymphe	Serum	Herz	Leber	Gehirn	
5	16,5	20,5	10,01	0,04	0, 6	0,162	γ/ml bzw. g
1	8,8	8,8	1 17	0,15	0,16	0,018	γ/ml bzw. g

Abb. 3. Vergleich des Kanamycingehaltes verschiedener Organe (250 mg Kanamycin/kg)

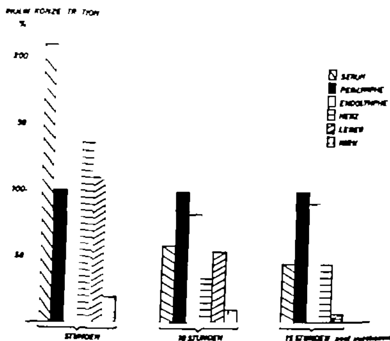


ABB. 1 Vergleich der Inulinkonzentration in Serum, Innenohrlymphen und Organen, bezogen auf Inulinkonzentration der Perilymphe = 100

essante Parallelen zu dem Verhalten der Antibiotica der Oligosaccharidgruppe, die als Struktureinheit ebenfalls einen Zucker aufweisen (Streptomycin Kanamycin Neomycin Framycetin Gentamycin und einige weniger bekannte Verbindungen) Diese Zuckerantibiotica sind ebenfalls nicht resorbierbar und konnen nicht sezerniert werden als Substanzen des extrazellularen Raumes konnen sie nur durch Diffusion und Filtration in die Innenohrflüssigkeiten gelangen Bei gleicher d. h aequimolarer Dosierung *und im niedrigen, toxisch nicht wirksamen Dosisbereich* verhält sich Kanamycin sehr ahnlich wie Inulin Seine Konzentration in der Innenohrlymphe ist außerordentlich hoch und übertrifft diejenige anderer Organe und die des Blut bzw Serumspiegels um ein Vielfaches. Seine Verweildauer ist ungewöhnlich lang (Stupp)

Noch deutlicher kommt dieser Kumulationsprozeß bei der für tierexperimentelle Untersuchungen üblichen ototoxischen Dosierung von 250 mg pro kg zum Ausdruck

Die Höhe der Konzentration und die Dauer der Einwirkungszeit im Innenohr sind u E für die spezifisch ototoxische Wirkung von ausschlaggebender Bedeutung

Im Gegensatz zu dem Verhalten der Zuckerantibiotica liegt die Konzentration des Penicillins stets deutlich unter der des Blutspiegels, Tetracycline erreichen überhaupt keinen wirksamen Innenohrspiegel auch wenn sie in allen anderen Organen in hoher Konzentration nachweisbar sind. Tetracycline können bekanntlich die Blutliquorschranke nicht überschreiten das Gleiche gilt offenbar für die Blut Innenohr Lymphschranke (Stupp)

nen, die aber im Vergleich mit den sonst in anderen Organen, wie Herz, Leber oder Gehirn vorliegenden Konzentrationen, wie sie vorhin dargestellt waren, doch noch relativ hoch sind.

Bemerkenswert ist, daß der Streptomycinsulfatblutspiegel zwischen 2 und 5 Stunden nach der Injektion nur wenig, und zwar von 24 γ/ml auf 20 γ/ml abnimmt, während die Perilymphkonzentration von 10 γ/ml auf über 20 γ/ml ansteigt. Würde man noch länger, 12 oder 24 Stunden, warten, so käme diese Tendenz, eine Abnahme des Blutspiegels bei zunehmender oder gleichbleibender Innenohrkonzentration, noch deutlicher zum Ausdruck. Derartige Untersuchungen werden zur Zeit durchgeführt.

Auffällig sind ferner bei diesen Versuchen am Menschen die ungewöhnlich großen Schwankungen der Perilymphkonzentrationen bei den einzelnen Versuchspersonen, die eine statistische Auswertung sehr erschweren. Die Blutspiegel waren dagegen weitgehend konstant, so daß eine renale Ausscheidungsstörung als Ursache ausgeschlossen werden kann. Trotz niedriger Dosierung überraschten in einigen Fällen ausserordentlich hohe Perilymphspiegel, die die Blutkonzentration um ein Vielfaches übertrafen. Man muß sich fragen, ob die bei einigen Menschen vorkommenden extrem hohen Streptomycinkonzentrationen im Innenohr für die in der Klinik gelegentlich schon bei kleiner Dosis völlig unerwartet auftretenden und daher sehr gefürchteten Innenohrschädigungen verantwortlich gemacht werden können. Damit könnte möglicherweise ein Phänomen erklärt werden, für das wir bisher nur die Bezeichnung „individuelle Überempfindlichkeit" hatten.

SUMMARY

In earlier examinations on guinea pigs it was shown by Stupp that the aminoglycosidantibiotics (streptomycin, kanamycin, neomycin) obtain a very *high* concentration and a very long durance in the peri- and endolymph. The same effect has now been proved in the perilymph of men with streptomycin injected in a therapeutic dose.

RÉSUMÉ

Stupp a déjà démontré par des expériences faites sur des cobayes que les aminoglycosides-antibiotiques atteignent une concentration très élevée et de longue durée dans la péri- et endolymphe. Le *même effet* est maintenant aussi révélé dans la perilymphe de l'homme en cas d'une injection streptomycine dans une dose therapeutique.

Dr. med. A. Meyer zum Gottesberge
Moorenstrasse 5,
Düsseldorf, Deutschland

DISCUSSION

J. Friedmann: We have referred to the paradox phenomena observed in experimental animals and in infants: catheters, i.e. lower dry causing greater damage in

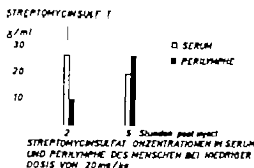


ABB. 4 Streptomycinsulfatkonzentrationen in Serum und Perilymphe des Menschen bei niedriger Dosis von 20 mg/kg

bessernden Operation (Stapedektomie) Perilymphe zu entnehmen In zahlreichen anderen Fällen scheiterte die Perilymphabnahme beim Menschen meistens infolge zu starker Blutungen Diese Versuche wurden von vornherein verworfen Für diese Untersuchungen wurden nur einwandfreie Perilymphen ohne Blutbeimengungen benutzt

Die Patienten erhielten eine einmalige Injektion von Streptomycinsulfat in einer normalen therapeutischen Dosierung von 20 mg/kg und zwar eine Gruppe 2 Stunden vor der Perilymphabnahme und eine andere Gruppe 5 Stunden vor der Entnahme.

Die Lymphe wurde nach vorheriger sorgfältiger Blutstillung mit Hilfe feinster selbstgefertigter Glaskapillaren aus einer kleinen Perforation in der Steigbügelfußplatte entnommen Gleichzeitig wurde Venenblut abgenommen das Serum abzentrifugiert und die Proben Serum und Perilymphe bis zur Durchführung der bakteriologischen Untersuchung eingefroren.

Die Bestimmung des Antibioticagehaltes erfolgte auf bakteriologischem Wege durch Messung der antibiotischen Aktivität Es wurde das allgemein in der Bakteriologie übliche Agar Diffusions-Verfahren angewendet und zwar bei Serum und Gewebe in Form des Loch Testes und bei Perilymphe mit Hilfe des Blättchen Testes. Verwendet wurden Filterblättchen mit einem Durchmesser von 5 mm Als Teststamm diente B subtilis ATCC 6633 Die niedrigste feststellbare Streptomycinkonzentration betrug im Serum 0 01 γ/ml bzw 0 08 γ/g in der Perilymphe dagegen 0 5 ml

Während bei den tierexperimentellen Untersuchungen in der Regel höhere Dosen und vor allem toxischere Antibiotica wie Kanamycin verwendet wurden mußten wir uns bei den jetzigen Versuchen am Menschen um jegliches Risiko zu vermeiden, auf möglichst niedrige Dosen und das am wenigsten ototoxische Antibioticum dieser Gruppe das Streptomycinsulfat, beschränken In früheren Arbeiten hatten wir schon eingehend zeigen können daß die Kumulation bei höheren toxischen Dosierungen und vor allem bei den toxischeren Substanzen wie Neomycin weitaus am ausgeprägtesten ist

Erwartungsgemäß zeigen die Streptomycinsulfatspiegel in der Perilymphe im therapeutischen Bereich beim Menschen nur mäßig hohe Konzentratio-

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REGIONAL DIFFERENCES IN SENSITIVITY OF THE VESTIBULAR SENSORY EPITHELIA TO OTOTOXIC ANTIBIOTICS

H. H. LINDEMAN

From the Otolaryngologic 1 Department of the University of Gothenburg Gothenburg Sweden

Th effect of intratympanic application of streptomycin nd parenteral i jection of kanamycin upon the estibular sensory epithelia have been studied with the surf ce specimen technique. Clear regional differences vulnerability to these ot t xic ntibiotics hav been noticed. The sensory epithelia of the cristae amp llares were more vulnerable than th macula utriculi, which in turn wa distinctly more vulnerable than the macula sacculi The degeneratiy hanges involved primarily the central part of the crista epithelium and the striola of the maculae.

Antibi tics f the strept mycee group have side effects, one of the most serious f which is a toxic action upon the mechanisms of hearing and equilibrium Since Berg (1949) and Caussé (1949) first observed degenerative changes in the vestibular sensory epithelia and in the organ of Corti of treptomycin treated animals, there has been general acceptance of the inner ear sensory epithelia as the primary sites of these toxic effects.

Recent studies have revealed a specific pattern of degeneration in the rg n of Corti following administration of ototoxic antibiotics (Beck & Krahl 1962 Hawkin & Engström, 1964 Kohonen, 1965) Differences in sensitivity to treptomycin among the cristae and the maculae and even within each crista, ha e been described by some authors however the results are to some extent controversial

By the aid f light and phase contrast microscopic studies of surface preparations from the vestibular sensory regions, clear regional differences in tructure ha e been demonstrated (Lindeman, 1969) Against this background the present study was carried out in order to analyze the overall pattern of damage in the vestibular sensory epithelia following administration of totoxic antibiotics. A short communication of some of the present findings ha been published (Lindeman, 196)

MATERIAL AND METHODS

The ubject f the experiment were 20 guinea pigs (non-albinos) of 2o0-350 g body weight and having a normal pinna reflex The animals were divided into two groups

Th ork as supported by the Norwegia Research Council for Science d the H ma ities nd by the Physiological P ychology Branch, Office of Na l Research, Washi gton D.C. unde Contract N 62558 4961 with H Engström.

individual animals, and vice versa Regenerative or reparative fissures play an important role in the end-result.

F Escher I would like to mention the experiments on monkeys in the Bernese clinic by M Neiger It could be proved that the concentration of streptomycin in the perilymph is rising during 24 hours and retained inside as in a prison. This is also a confirmation of the results of Mr Meyer z Gottesberge

A Meyer zum Gottesberge (Antwort) Unsere Untersuchungen erklären sicherlich nicht alles und nicht die Vorgänge in den Zellen Aber wie Paracelsus sagt, liegt der Unterschied ob ein Ding ein Gift sei oder nicht in der Dosis, d h. in der Konzentration

Acta oto-laryngologica 67: 177–189 (1969)

REGIONAL DIFFERENCES IN SENSITIVITY OF THE VESTIBULAR SENSORY EPITHELIA TO OTOTOXIC ANTIBIOTICS

H. H. LINDEMAN

From the Otolaryngological Department of the University of Gothenburg, Gothenburg, Sweden

The effect of intratympanic application of streptomycin and parenteral injection of kanamycin upon the vestibular sensory epithelia have been studied with the surface specimen technique. Clear regional differences in vulnerability to these ototoxic antibiotics have been noticed. The sensory epithelia of the cristae ampullares were more vulnerable than the macula utriculi, which in turn was distinctly more vulnerable than the macula sacculi. The degenerative changes involved primarily the central part of the crista epithelium and the striola of the maculae.

Antibiotics of the streptomyces group have side effects, one of the most serious of which is a toxic action upon the mechanisms of hearing and equilibrium. Since Berg (1949) and Caussé (1949) first observed degenerative changes in the vestibular sensory epithelia and in the organ of Corti of streptomycin-treated animals, there has been general acceptance of the inner ear sensory epithelia as the primary sites of these toxic effects.

Recent studies have revealed a specific pattern of degeneration in the organ of Corti following administration of ototoxic antibiotics (Beck & Krahl, 196 ; Hawkins & Engström, 1964; Kohonen, 1965). Differences in sensitivity to streptomycin among the cristae and the maculae and even within each crista, have been described by some authors; however, the results are to some extent controversial.

By the aid of light and phase contrast microscopic studies of surface preparations from the vestibular sensory regions, clear regional differences in structure have been demonstrated (Lindeman, 1969). Against this background the present study was carried out in order to analyze the overall pattern of damage in the vestibular sensory epithelia following administration of ototoxic antibiotics. A short communication of some of the present findings has been published (*Lindeman, 1967*).

MATERIAL AND METHODS

The subjects of the experiments were 20 guinea pigs (non-albinos) of 250–350 g body weight, and having a normal pinna reflex. The animals were divided into two groups.

This work was supported by the Norwegian Research Council for Science and the Humanities and by the Physiological Psychology Branch, Office of Naval Research, Washington D.C., under Contract N 62558 4284 with H. Engström.

1 In 15 animals a varying dose (5–20 mg) of streptomycin sulphate, dissolved in 0 1 ml Ringer fluid was injected through the tympanic membrane into the middle ear of one side under nebumal anaesthesia. The other ear served as control Four–five hours after the injection, the animals showed a falling tendency towards the operated side, spontaneous nystagmus towards the opposite side, and ataxia. The *pinna reflex disappeared.* The vestibular symptoms were considerably reduced *after a few days*, but during *the whole* period of observation the animals showed ataxia. The animals were sacrificed 2–8 days after the injection

2 Five animals were treated by parenteral injections of kanamycin sulphate 300 mg/kg daily over a period of 8–15 days. None of the animals revealed any apparent vestibular symptoms The animals were sacrificed 5–15 days after completing the treatment

The temporal bones of the guinea pigs were fixed/stained in 1.5% osmic acid solution or fixed in a solution of equal parts of methanol and ether and stained with Giemsa solution Five temporal bones were embedded in epon and sectioned for phase contrast microscopy In the other temporal bones, the vestibular sensory epithelia were dissected free for light and phase contrast microscopic studies with a technique described by Lindeman (1969) The organ of Corti was studied using the surface specimen technique (Engstrom *et al* 1966)

OBSERVATIONS

After both streptomycin and kanamycin administration, degenerative changes could be observed in the inner ear In the vestibular part these degenerative changes were restricted almost completely to the sensory cells. Changes in the sensory cell nuclei were usually the first signs of damage to the epithelium, as studied in light microscopy (Fig 1) In some specimens a moderate swelling of the nuclei could be observed, the nuclei appearing larger and fainter than normally More frequently agglomeration and displacement of the chromatin towards the periphery of the nucleus of the sensory cell could be seen Furthermore, pycnotic and fragmented nuclei, staining strongly with Giemsa, were observed Before disintegration of the nuclei, a disarrangement of the sensory hairs was seen The hairs spread out on the surface of the epithelium and often clumped together (Fig 2) Protrusions of the cytoplasm of the sensory cells into the endolymphatic space, occasionally seen in normal animals, occurred frequently in the experimental animals A final stage was the so called "collapse figure indicating a complete degeneration of the sensory cell (Fig 1) The collapse figures were easily identified and permitted exact quantitative estimates of sensory cell loss After a few weeks, however the supporting cells surrounding the degenerated *sensory cell filled up the gap of the previous sensory* cell, and the collapse figure disappeared This was a stage comparable with a "phalangeal scar" in the organ of Corti (see Engström *et al* 1966) In

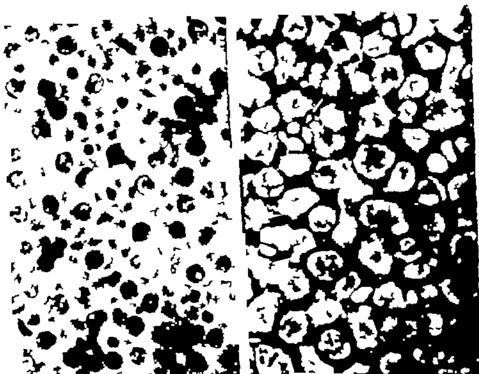


Fig. 1 Surface preparations of the vestibular sensory epithel f the gui pig fter administration of streptomycin. ( ) Macula triculi showing nuclei f both normal and degenerati g sensory cells. Clumpi g of the hromatin, pycnosis, and fragment tion f the nclei illustrat different degenerativ stages. Giemsa stai ing   757 (*b*) Sensory epithelium of crista mpullari revealing parti l los of sensory cells. The collapse f gure (rrow) indicates complet degeneration of   sensory cell. Osmic acid f xation 1515

c ntrast, whereas in the organ of Corti the position of a lost sensory cell m y be easily bserved due to the regular cellular pattern, it was more difficult to localize the former position of a sensory cell in the vestibular sensory epithelia, a the cellular pattern in these regions is normally rather irregular Lindeman, 1960)

Other changes could also be observed in the degenerated vestibular sensory epithelium. After a loss of about 50% of the sensory cells, a reduc tion in height of the epithelium was noticed In addition, an increased amount f osmiophilic granules could often be seen

A D generation pattern following application of strept mycin into the middle ear

The control ears w re all normal Degenerative changes in the vestibular apparatu as well as in the organ of Corti were noticed in all the operated ears. The extent of these hanges could be related to a certain degree to the am unt of streptomycin injected Animal treated with large doses of strep-

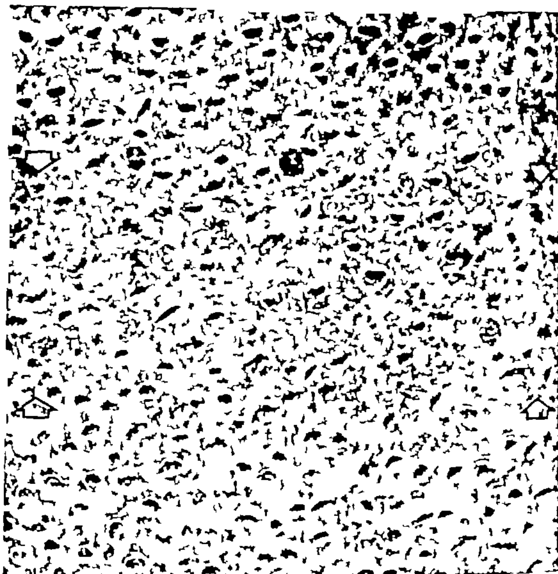


FIG. 2. Surf ce preparation from macula ut iculi of a guine pig posed to ka m cin Th hair bundle of th sensory c lls are seen In th tri la (*betwe n arr ws*) most of th hair bundles are lost r ppear different f m n rm l Osmic acid fi ati 96.

tomycin showed early and extensive degenerative changes. Thus, only 2 1/2 days after treatment with massive doses of streptomycin a considerable loss of vestibular and cochlear sensory cells could be noticed Animals treated with smaller doses showed less pronounced morphological changes in most cases, and these developed more slowly

The degeneration of the vestibular sensory epithelia followed a specific pattern The sensory epithelia of the crista ampullares were more vulnerable than the macula utriculi, which in turn was clearly more vulnerable than the macula sacculi Furthermore, distinct regional differences in sensitivity were apparent within each sensory region

On both the macula sacculi and the macula utriculi, the degenerative changes involved the striola primarily Only when the loss of sensory cells



Fig. 3. Same macula utricul as I Fig. 2. The microscope I focused on the epithelial rface I the st l la. Some collapse fig res (ll and) re seen. Arrow indicat direc-tio of morphological polarization f se sory cells, where basal bodies may be identi-fied. Direction of polarization is reversed the middle of the striola 1560.

In this region wa exten ive were degenerative changes observed in the periphery of the pithelium On the cristae the central regions of the sen sory epithelia were more vulnerable th n the periphery All three cristae showed about the same extent and pattern of degeneration

A seen in light microscopy the supporting cells of the vestibular sensory epithelia appeared normal, as did the cupulae and the statoconium mem branes.

In addition to vestibular changes, damage to the organ of Corti was observed. The basal coils of many cochleas showed a complete loss of outer hair cells, and often a degeneration f Deiters cells and pillar cells as well The degeneration of outer h ir cells gradually decreased towards the apex, wherea an increasing number of degenerated inner hair cells were observed. The uter hair cell howed a gradient of decreasing vulnerability from the first t the third row

B *Degeneration pattern following parenteral injections of kanamycin*

F ur out of five animals in this group revealed degenerations of the inner ear sensory cells. In two animal the macula sacculi ppeared grossly nor mal, the only degenerati e changes being found in the striola of the macula utriculi and in the central region of the crista (Figs. 2, 3 and 4) Two nimal howed slight los f sensory cells in the striola of the macula

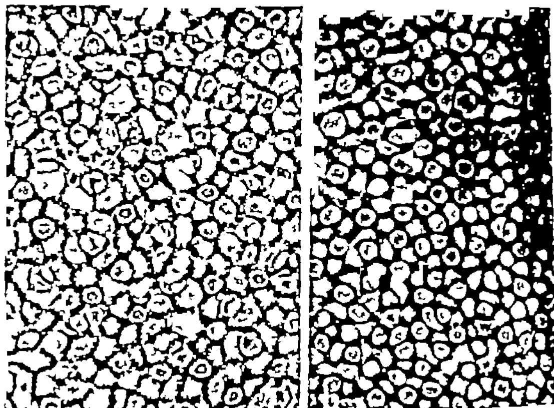


FIG. 4 Two photomicrographs from the same crista ampullaris of a guinea pig treated with kanamycin. (*a*) In the central area a high percentage of the sensory cells are degenerated and appear as collapse figures (*arrows*). (*b*) In the periphery the sensory epithelium looks completely normal, with the exception of one single degenerated cell (*arrow*). *a* and *b* × 764.

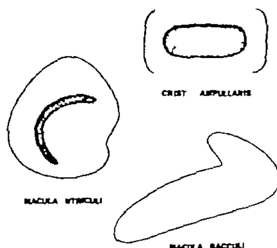


FIG. 5 Diagram illustrating the regional differences in vulnerability to ototoxic antibiotics. The density of dots indicates the relative sensitivity of the sensory cells in the different areas. The sensory epithelia of the cristae are more vulnerable than the macula utriculi, which in turn is [illegible]ly more vulnerable than the macula sacculi. The central areas of the cristae and the striola of the maculae are more sensitive than the peripheral parts.



Fig. 6. Surface preparations of organ of Corti from guinea pig treated with kanamycin. (a) T[illegible] coil from base. All inner hair cells (*IHC*) intact (the hairs of the cells are in focus). Total loss of outer hair cells in the first row (*1*), partial loss in second (*2*) and third (*3*) rows. (*b*) Two and three-quarter coil from base. Somewhat longer exposure time to kanamycin than in *a*. All inner hair cells, and all sensory cells in the first and second row degenerated. Only one degenerated sensory cell in the third row. *a* and *b* ×1070.

sacculi. In these animals a considerable loss of sensory cells both in the striola of the macula utriculi and the central regions of the cristae, and a smaller loss of cells in the peripheral parts of these sensory epithelia were observed.

The intratympanic application of streptomycin and parenteral injection of kanamycin gave the same pattern of degeneration in the vestibular sensory regions. The relative vulnerability of the sensory cells in the different areas is illustrated in Fig. 5. The sensory cells of type I were more sensitive than those of type II. Among the type I cells, those located in the central regions of the cristae and the striola of the maculae were the most vulnerable.

The degeneration pattern in the cochlea was similar to that seen after streptomycin administration (Fig. 6); however, whereas the sensory cells on the cristae ampullares and the macula utriculi generally were more sensitive to streptomycin than the hair cells of the organ of Corti, the latter were generally more sensitive to kanamycin than the vestibular sensory cells. No apparent changes of the statoconium membranes and the cupulae could be seen.

DISCUSSION

The sensitivity of the sensory epithelia of the inner ear to ototoxic antibiotics is different in different species. Thus it appears from the literature that equivalent doses of, e.g., streptomycin produce more pronounced damage in man and cat than in the guinea pig. In fact, in the guinea pig it is difficult to induce substantial damage to the inner ear sensory epithelia by parenteral application since most of the animals die as a result of general intoxication before such damage can be observed. However, massive degenerative changes in the inner ear can be produced by instilling streptomycin into the middle ear. Spoendlin (1966), using this avenue of administration in the cat, showed that the effect upon the vestibular sensory epithelia was not a result of non-specific osmotic changes, but depended upon a direct toxic influence of the streptomycin. In his opinion the streptomycin diffused from the middle ear via the round window membrane into the inner ear.

As early as 12 hours after intratympanic injection Spoendlin (1966) observed, by electronmicroscopy, an agglomeration of chromatin in the nuclei of the type I cells. Later, loss of ribosomes and endoplasmatic reticulum became apparent, whereas mitochondria and membranes of nuclei and cells remained normal for some time. The nerve endings and the nerve fibers were also found to be normal. In Spoendlin's opinion the streptomycin attacked primarily the protein synthesis, which is dependent upon the cell nuclei and the ribosomes. On the other hand Wersäll & Hawkins (1962) after parenteral streptomycin injections in the cat observed changes of the mitochondria, swelling and vacuolization of the cytoplasm of the sensory cell prior to pycnosis of the nuclei. Studying the effect of parenteral injections of streptomycin in guinea pig Duvall & Wersäll (1964) postulated that the streptomycin affected primarily the plasma membrane of the cells and the mitochondria.

In the present investigation it was difficult to determine the exact sequencies of degeneration, as the finer intracytoplasmic changes could not be visualized in light microscopy. Early signs of degeneration were agglomeration of the chromatin in the periphery of the nucleus and sometimes a slight swelling of the nucleus. Later the nuclei became pycnotic or fragmented. Before complete disintegration of the nuclei occurred, changes in the hairs could be noticed. Furthermore, an increased number of cytoplasmic protrusions were seen which were similar to those described by Wersäll & Hawkins (1962) and Spoendlin (1966). A final stage was the appearance of the collapse figure. The formation of the collapse figures in the vestibular sensory epithelia corresponded to those described in the organ of Corti (Engström *et al.* 1966).

Some authors have reported regional differences in the sensitivity of the vestibular sensory cells to streptomycin. In the present study a distinct gradation in the sensitivity of the different areas was evident after both

intratympanal application of streptomycin and parenteral administration of kanamycin. In agreement with findings by McGee & Olszewski (1962) and Spoendlin (1966) the sensory cells of the cristae were found to be more vulnerable than those of the maculae Igarashi (1965) and Igarashi *et al* (1966) stated that the administration of streptomycin sulphate is a useful method of selectively suppressing the semicircular canal function" and they described a nearly selective damage to the sensory cells of the cristae in squirrel monkey Such findings, however were not supported by the present study in guinea pig, as extensive damage to the cristae epithelium wa always accompanied by a considerable loss of hair cells in the macula utriculi. Duvall & Wersäll (1964) did not notice any difference in vulnerability between the sensory cells of the crista and those of the macula utriculi in the guinea pig.

In the present study the macula utriculi always showed more extensive changes than the macula sacculi. This is in agreement with observations by Berg (1951) Rüedi *et al* (1951) Hawkins *et al* (1952) and Wersäll & Hawkin (1962)

Many authors have described a more pronounced loss of hair cells on the t p of the crista than on its slopes (Berg, 1951 Rüedi *et al* 1951 Hawkin *et al* 1952 Wersäll & Hawkins, 1962 McGee & Olszewski, 1962) a finding which is not in agrement with the present observations. As discu sed by Lindeman (1969) it seems justified to divide the sensory epithelium on the crista int central and peripheral regions Thus the peripheral part f the sensory epithelium located on the top (*vertex*) *of the crista* and b rdering the plana semilunata, morph logically as well as with respect to ulnerability to streptomycin nd kanamycin corresponds to the peripheral part f the epithelium on the slopes of the crista. In the present study the sensory cells in the central region were more vulnerable than the more peripherally located cells

Neumann & Neubert (1958) described regional differences in sensitivity within the macula utriculi. They observed degenerative changes which tarted in the *central part* of the macula utriculi however in th ir opinion, this region did not correspond to the *striola*.

Both after parenteral and intratympanic application of streptomycin in the cat the type I cell has been reported to be more sensitive than the type II cell (Wersall & H wkins, 1962 Spoendlin, 1966) On the other hand Duvall & Wersäll (1964) did not find clear differences in sensitivity f the type I and type II cells in the guinea pig In the present investigation, the type I cell seemed in general more vulnerable than the type II cells. Most sen itive were those type I cell in the central parts of the cristae and in the triola f th maculae Again t the background of the highly sepcial ized tructure of these regions (see Lindeman, 1969) this observation is most interesting. It support th suggestion that the function of these regions differs from that f the peripheral parts. It is not yet possible to explain why th cell in the central parts f the cri te and in the striola of the

maculae are particular sensitive to ototoxic antibiotics however according to Spoendlin (1966) *there seems to be a certain correlation between the intensity of the protein metabolism of the sensory cells and their sensitivity to streptomycin*

In the present study the sensory cell loss in the least damaged epithelia was confined to the central parts of the cristae and the striola of the maculae In future studies of vestibular sensory epithelia, it must be realized that degenerative changes may be restricted to such small areas. In the case of a macula with damage restricted to the striola, a longitudinal section through the striola, will *give an exaggerated picture of the extent of damage* while an extra striolar section might fail to show any degeneration at all It is also important to know that in a medially situated part of the macula utriculi, the number of sensory cells per unit of area is *less* than elsewhere peripherally (Lindeman, 1969) Thus a section through this region may give an erroneous impression of sensory cell loss.

Some authors have tried to explain the vulnerability of the sensory regions in the inner ear to different noxious agents on the background of phylogeny In a study of pathological types of cochleo-saccular degeneration" Schuknecht *et al* (1965) concluded "In demonstrating a greater susceptibility to injury the pars inferior of the labyrinth abides by the biological rule that *the phylogenetically newer system in an organism generally exhibit a* distinctly greater susceptibility to abuse than do older systems " The results of the present study however fails to confirm that statement. Thus, the macula sacculi which together with the cochlear duct represents the phylogenetically youngest part of the labyrinth, turned out to be most resistant to both kanamycin and streptomycin It must be emphasized that the degeneration pattern after exposure to noxious agents is dependent upon the specificity of the agent In the present investigation, the hair cells of the organ of Corti were generally more sensitive to kanamycin than the vestibular sensory cells The sensory cells of the cristae and the macula utriculi on the other hand were generally more sensitive to streptomycin than were the cochlear hair cells Furthermore Winther (1968) after X ray irradiation of the inner ear observed a different pattern of degeneration in the vestibular sensory regions than that reported here

Berg (1951) reported disappearance of the statoconium membrane after streptomycin intoxication a phenomenon also noted by Kimura & Perlman (1956) after obstruction of the v cochleae inferior They explained the breaking down of the statoconium membranes as a result of extensive changes of the sensory epithelium In the present investigation no convincing degenerative changes of the cupulae or the statoconium membranes could be noticed however the possibility that the degenerative changes were too small or the observation period too short must still be held open

RESUME

L effet d pplicati n intratympanale de streptomycine et d'injectio s parentérales de kanamyci e sur l'épithélium sensoriel estibulaire a été étudié en se servant d la technique de préparation de la surface spécimen. Variation régionales de la sensibilité sont alors trouvées. Les changements dégénératifs se manifesta ent premièrement dan les parties centrales de l'épithélium des cristae et d n la striola des maculae.

ZUSAMMENFASSUNG

Die Wi kung von i tratympanaler Streptomycin pplikation und parenteralen Kanamycininjektionen auf den estibularen Sin esepithelien ist mit der Häutchentech ik studiert worden. Dabei sind klare regionale U terschiede in der Vulnerabilität gegenüber den oben genan ten ot t xischen A tibiotika gefunden worden. Die Sinnesepithelien der Cri t e amp llares waren mehr vulnerabel als die Macula utriculi und diese wiederum deutli h mehr ulnerabel als d e Macula sacculi. Die degenerativen Verä derungen zeigten sich hauptsächlich in den zentralen T ile des Crist epithels u d in der Stri la der Maculae.

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DISCUSSION

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M Portmann Très intéressé par cette communication ma question est la suivante Pensez vous qu'il-y-a une relation entre les zones lesées et les zones de fonction maximum des macula? Par exemple dans l'organe de Corti ce sont les cellules externes du premier Tour qui sont les plus affectées c'est à dire celles qui sont les plus stimulées par les vibrations. La toxique en bloquant le système enzymatique cellulaire fragile donc la cellule vis à vis du stimulus. On peu donc supposer que la localisation n'est pas specifique du toxique mais specifique du fonctionnement

H Engström Mr Kelemen has touched upon a most important problem, that is, the question of normal and pathological In guinea pigs the inner ear is extremely regular in structure, but this is not always true for monkeys, and for human beings over 30–40 there is always a rather pronounced irregularity

H H Lindeman (Reply) to Mr *Friedmann* The collapse figures have been described in the organ of Corti by Engstrom *et al* (1966) The mechanism in the formation of the collapse figures in the vestibular sensory epithelia seems to be similar After disintegration of the cytoplasm and the cell nucleus, the plasma membrane collapses" and loses its attachments to surrounding supporting cells, except in certain areas, where the attachments are especially tight This gives the characteristic spider like appearance of the collapse figure as seen on surface preparations.

Of course I agree with you that the surface preparation technique is not the only way to study inner ear sensory epithelia. A combination of different methods is always superior to the use of one single method However for certain types of investigations, the surface preparations are especially well adapted. The method is fast and reliable It allows identification of virtually every sensory

and supporting cell within a sensory region, nd is, therefore useful for quanti tati e studies. The method also permits exact orientation, which is a prerequisite for systematic studies of corresponding region in different animals.

T Mr *Kelemen* Before studying th effect of n xious agents upon the estibular sensory epithelia, the structure of these epithelia was studied in more than 200 normal guinea pigs, a backgrou d which was of great help in identifying abnormalities which appeared after treatment with otot xic ntibiotics.

T Mr *Portmann* The clear regi n l differences in the normal structure of the estibular sensory epithelia, in addition to the regi nal differences in sensitivity t ototoxic ntibiotics, strongly suggest a different fu ction of th central and peripheral areas of the crist e and maculae H wever as far as I know no functional studies have been made with thi suggestion in mind, nd it is therefore not possible to draw any con lusions regarding thi matter at the moment.

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Simplex virus, reovirus, and unidentified infectious particles, has suggested a viral aetiology in Burkitt's lymphoma. In addition Burkitt's lymphoma cells have been successfully identified in cultures used to detect antibodies in human serum. In view of these discoveries, it is conceivable that nasopharyngeal carcinomas might be similarly caused by a virus.

The purpose of this communication is to report on the results of preliminary studies on viral isolation and tissue culture from material obtained from nasopharyngeal carcinomas in Chinese patients. Serological investigations and epidemiology are also considered.

Tissue cultures

Little information is available in the literature on tissue culture of nasopharyngeal carcinomatous material.

Burke (1967) in Singapore, submitted five specimens obtained from primary growths of nasopharyngeal carcinoma to tissue culture. He was able to maintain all growths and multiplication of explants *in vivo* for a maximum of 35 days, with an average survival time of 30 days. The four metastatic cervical lymph node tissues all failed to grow. All the five cases showed spontaneous degeneration followed by death. It is emphasized that the demonstration of viruses in human tumour tissues does not necessarily mean a causal relationship.

Present Series

Tissues obtained from the primary growth of 10 histologically proven and untreated epidermoid carcinomas of the nasopharynx were sent to Dr J Huebner for tissue culture and viral isolates. Only one specimen survived in culture for a short period. It is most likely that distance and difficulties in transportation and delivery contributed to this failure as the nasopharyngeal carcinoma cells did not arrive in good condition. Improved packing and more reliable shipping procedures are under consideration. This line of research will be continued.

Table 1 gives the adenovirus incidence demonstrated serologically amongst chinese nasopharyngeal carcinoma patients compared with the

TABLE 1 *Comparative findings of adenovirus studies*

	Hong Kong virus isolates (1966–1967)	Serological positives (N.P.C.)	Total no. of cases	Percentage
Type adenovirus				
2	4	6	47	12.77
3	10	0	47	—
5	1	0	47	—
7	3	19	47	40.43
12	0	8	47	17.02

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VIRUS AS THE CAUSE OF RHINOPHARYNGEAL CARCINOMA

D LAING

Hong Kong

Tissue cultures from the primary growths of 10 nasopharyngeal carcinoma patients failed to grow Nasopharyngeal carcinoma patients in Chinese yielded more evidence of infection in adenovirus types 7 and 12 than the normal Chinese population Sera from 27 Chinese nasopharyngeal carcinoma patients were tested for precipitating antibody to antigen prepared from Burkitt s lymphoma cells. There was a high incidence 85% of positive reactions in these patients. The possibility that nasopharyngeal carcinoma has a viral cause or relationship with Burkitt s lymphoma is discussed. While there is no evidence to support this contention the possibility can not be excluded of a predisposing virus infection as the initiator of a malignant process or transformation.

I have been asked to express my views on the viral aetiology of rhinopharyngeal carcinoma Let me say at once I am not a virologist and my knowledge of this subject is extremely limited, but my interest in the study of the possible connection of viruses in this neoplasm has been stimulated not only by the dearth of information relating to it but also by the failure to find a known cause for this unique and recalcitrant growth

In the past there was wide acceptance of the hypothesis that inhaled exogenous, environmental carcinogen was an important aetiological factor However there was insufficient scientific evidence to support this contention since no known offending carcinogen has been found The ethnic factor in this disease has received increasing recognition because of the characteristic epidemiological features and high incidence in the Mongoloid races of South East Asian countries and related communities In addition, this disease predominates in all communities where Chinese congregate outside their homeland

Considerable and rapid advances in virology have been made since the pioneering discoveries of Ellerman & Bang (1908) five decades ago The relationship between viruses and tumours has been extensively studied by many virologists whose significant and classical contributions, in combination with the discovery of tissue culture and the development of electron microscopy have made it possible to isolate and study the structure and properties of viruses and investigate intracellular viral behaviour and events

At present, no human cancer has yet been proved to derive from any virus, but two human adenoviruses, types 12 and 18 have been found to produce tumours in hamsters and mice Recently the isolation of Herpes

TABLE 3. *Comparison of results with Old et al*

Author	Positive sera		
	Burkitt lymphoma	N.P.C.	Other carcinoma and healthy controls
Old *et al.*, 1966	56	*African* 74	11 12
		U.S.A 95	
This series, 1968		*Chinese* 83	

series is concerned only with southern Chinese and represents the first observation of its kind relating to this serological overlap

The incidence is 83% compared to Old's African figure of 74% and north American of 95% positives for this precipitating antibody as compared to a control incidence of between 11 and 12% It is suggestive of a serological kinship between Burkitt's lymphoma and nasopharyngeal carcinoma, but, as to what this exact relationship is, only further serology study can help

It is possible that this high incidence of positive sera in patients with Burkitt's tumour and in nasopharyngeal carcinoma in the Chinese may not be confined to these two neoplasms.

All the 27 cases were histologically proven to be epidermoid carcinomas of the primary growth. When the blood samples were taken, the condition of all these cases was quiescent there was no local recurrence, palpable cervical lymph node or distant metastases. Their survival period ranged from 10 months to 8 years and 8 months

The four negative series survival period varied from 2 to 7 years. Only one of these received combined radiotherapy and chemotherapy Of the 23 positive sera, 10 were treated with radiotherapy only and 13 received combined radiotherapy and chemotherapy The survival period varied from 10 months to 8 years and 8 months. All the five adult control cases were negative.

From a study of this small number of cases, there was no correlation between stage of the disease, survival period, type of therapy and positive and negative sera.

Epidemiology

It has been demonstrated and accepted that the southern Chinese have a striking preponderance of rhinopharyngeal carcinoma. Ho's analysis of a large series of cases found that the frequency rates differed in different regions of South China the rates in Kwangtung are higher than those in

TABLE 2 *Preliminary complement fixation test*

Tumour antigen studies in 16 Chinese nasopharyngeal carcinoma patients

Tumours	Positive	Negative	No. of tests done
Adenovirus 7 #4	5	11	16
SV 40 #	1	10	11
Polyoma #3	1	6	7
Adenovirus 7 (FT 1160)	2	15	1
Polyoma tumour #4	7	16	18

adenovirus isolates in normal individuals in Hong Kong, carried out by the Government Microbiological Department during two years. The inference is that the nasopharyngeal carcinoma patients yield more evidence of infection than many of the Hong Kong population. All results which were "trace positive" were disregarded. If these were added, the evidence is further strengthened in adenovirus types 7 and 12, in each of which six "trace positives" were found.

Bearing in mind that no paired sera investigations were done, and comparing the few positive Hong Kong population isolates during the two years, and the definitely higher serological positives for adenovirus types 7 and 12 in nasopharyngeal carcinoma patients, more extensive studies into this aspect are indicated It is emphasized that the numbers are too small to draw any worth while conclusions.

In an earlier group 16 nasopharyngeal carcinoma patients sera were studied, using assorted tumour antigens by the complement fixation test. The results are equivocal and a particular feature was that where SV40 or adenovirus positives occurred among this early group positives also occurred in equal dilutions with normal rat cell clones and hamster cells also Other tumour antigens than the ones in the table yielded constantly negative findings for antibodies, among them being adenovirus 12 10 adenovirus 31 6 and respiratory syncytium virus So far all efforts to demonstrate any antibodies to tumour antigens have failed in the complement fixation test The significance is that this, at the present time indicates an unrewarding yield.

In view of recent findings that indicated a serological overlap between Burkitt s lymphoma and other tumours, a study of 27 Chinese nasopharyngeal carcinoma cases was made A search was undertaken for a precipitating antibody in nasopharyngeal carcinoma patients sera tested against Burkitt s lymphoma tumour antigen A positive precipitating antibody was found in 23 out of 27 of these patients, with four negatives, giving 85 % positive.

A comparison with Old *et al* (1966) is more interesting than this figure would suggest because Old drew the sera from Africans and patients in United States of America, but does not record results from Asiatics This

TABLE 5 *Chinese land and marine populations in Hong Kong in 1961 by age and sex*

Population	Under 25 yrs			25 yrs and over			All ages,
	M	F	M + F	M	F	M + F	M + F
Land	807,572	729,917	1,537 489	698,590	707,211	1 405,831	2,943,320
Marine	43,420	39,297	82,717	29,101	24,763	53,864	136,581
Total	850,992	769,214	1,620,206	727,691	732,001	1 459,693	3,079,901

in a population already known to have a high incidence of this disease and has led me to search for possible causal factors—environmental genetic or viral. Table 5 shows the Chinese land and marine population in Hong Kong in 1961 by age and sex. Table 6 shows a comparison between the incidence of nasopharyngeal carcinoma among the "boat people and the rest of Hong Kong.

Hong Kong has a marine population of 136,581 It is descended from mainland Kwangtung The people live mainly in their crafts of various sizes, earning their livelihood by fishing. Some are seamen and a minority have shore employment but continue to live in houseboats, where often two or three generations of a family share a common environment. The majority of this population is Tan ka and have been residents of Hong Kong for hundreds of years. They are highly religious and superstitious. They congregate in small communities in the various bays and fishing villages both in the Island and or the Kowloon Peninsula Intermarrying amongst these settlers is shown by the limited number of family names. The illiteracy rate is high Shore dwellers do not look favourably on mariage with the Tan ka s. During the last 10 years their standard of living has improved considerably The majority is physically healthy robust, and hardy The houseboat is kept scrupulously clean. Ventilation and natural light in the cabins are poor The floor area in each cabin varies *from* 30 to 40 sq ft. The "boat people spend most of their time on deck in the open-air where the atmosphere is anything but dusty Kitchen smoke is not an irritating factor a good outlet for fumes being *provided in the* kitchen. Butane gas i now used in the larger crafts. Incense smoke from the burning f joss sticks in the small alter is considerably less than in other homes because of economic reasons. Worshipping is generally done by the femal s. Neither inhalation of snuff nor opium is indulged in by these people Tobacco chewing is rare but the use of water pipes is common Di consists mainly of sea food, rice, small quantities of red and white meat, and large quantities of vegetables, and is roughly estimated at 3000 to 3500 calories daily A comparativ retrospective study of the ways of life of 15 nasopharyngeal carcinom cases amongst the Tan ka s with the same number from th land dwellers of more or less similar socio-eco-

TABLE 4 *Results of N P C patients sera for precipitating antibody to antigen prepared from cultured Burkitt s lymphoma cells*

No	Name	Age	Sex	Type of growth	Stage of disease	Radio-therapy	Chemo-therapy	Survival period	Result	Serology Positive	Serology Negative
1	L. S. N	43	M	Epidermoid ca.	3	yes	no	3 yrs 6 months	Deceased		
2.	F W K.	49	F	Epidermoid ca	2	yes	yes	2 yrs	Alive	±	
3.	L. Y L.	46	M	Epidermoid ca.	1	yes	no	7 yrs	Alive	+	
4	H. C. W	41	M	Epidermoid ca.	3	yes	no	3 yrs	Alive	+	
5.	L. S	33	M	Epidermoid ca.	3	yes	yes	1 yr 3 months	Alive	+	
6.	L. M H.	41	M	Epidermoid ca.	3	yes	yes	1 yr	Alive	+	
7	C. L W	39	M	Epidermoid ca.	3	yes	yes	2 yrs	Alive	+	
8.	N K	40	M	Epidermoid ca.	3	yes	yes	1 yr 6 months	Alive	+	
9	C. L. Y	30	F	Epidermoid ca.	3	yes	no	2 yr 8 months	Alive	+	
10	L F T	44	M	Epidermoid ca.	3	yes	yes	3 yrs	Alive	+	
11	W C.	47	M	Epidermoid ca.	3	yes	yes	2 yrs 3 months	Alive		
12.	M S. P	43	M	Epidermoid ca.	3	yes	yes	5 yrs	Alive	+	
13	S K H.	46	M	Epidermoid ca	2	yes	no	7 yrs	Alive		
14	Y B. T	32	M	Epidermoid ca.	2	yes	no	3 yrs	Alive	+	
15.	W Y	38	F	Epidermoid ca.	3	yes	yes	2 yrs	Alive	+	
16.	C. H.	42	M	Epidermoid ca	3	yes	yes	1 yr 3 months	Alive	+	
17	C. K W	31	M	Epidermolp ca.	1	yes	no	4 yrs	Alive		
18	L. S K	20	F	Epidermoid ca.	2	yes	no	3 yrs 10 months	Alive	+	
19	S C. T	60	M	Epidermoid ca.	3	yes	yes	2 yrs	Alive		
20	N P K.	39	M	Epiderm ld ca.	2	yes	no	3 yrs 9 months	Alive	+	
21	L. K. S	41	M	Epidermoid ca.	3	yes	no	2 yrs 11 months	Alive		
22.	L. H	42	M	Epidermoid ca.	2	yes	no	8 yrs 8 months	Alive	+	
23.	C K. W	55	M	Epiderm ld ca.	4	yes	yes	1 yr 8 months	Alive	+	
24	C. S	43	M	Epidermoid ca.	3	yes	no	8 yrs 3 months	Alive	+	
25	Y C. Y	30	M	Epidermoid ca	3	yes	no	3 yrs	Alive	+	
26.	K. Y K.	28	F	Epidermoid ca.	1	yes	yes	2 yrs	Alive	+	
27	L. K. S.	64	F	Epidermoid ca.	3	yes	yes	10 months	Alive	+	

the central provinces of *Chekiang Fukian and Kiangsu Further he showed* amongst the Hong Kong-domiciled group a significantly higher incidence in the floating population" which has a crude incidence rate of 124 per million as against 78 per million in the land population

This striking difference shows the existence of a localised risk community

Adenoviruses and Tumours

These are of particular interest, especially types 12 and 18, which were originally isolated from degenerated human adenoid tissue exhibiting spontaneous degeneration in culture. One of the properties of these viruses is, in some cases, that they cannot be recovered from the tumour they produced, and it has been suggested that the virus, after initiating a neoplastic conversion of the normal cell, disappears: a process analogous to the behaviour of the bacteriophages. Could this be the reason why the search for human tumour virus has been unsuccessful so far?

It is interesting to note that in spite of the frequency of upper respiratory tract infection in Hong Kong, especially in the low socio-economic group, adenovirus types 12 and 18 have not been isolated in 2 years, but eight adenovirus type 12 serological positives were found out of 47 nasopharyngeal carcinoma patients.

Another significant clinical finding is that in 6307 cases of nasopharyngeal carcinoma patients' case records, obtained from colleagues in Singapore, Taiwan, and Hong Kong, not a single one was adenoidectomized. As adenoidectomy is comparitively rarely performed on Chinese children, no connection or estimation can be made of its relation to the development of nasopharyngeal carcinoma. However, it may be a clue worth following up.

In the light of this very restricted research, it is not possible to express any conclusion on this complex problem, and further investigations will be done.

CONCLUSION

1. Tissue culture from the primary growth of 10 nasopharyngeal carcinoma patients failed to grow.

2. Chinese nasopharyngeal carcinoma patients yield more evidence of infection in adenovirus types 7 and 12 than the normal Chinese population.

3. Sera from 27 Chinese nasopharyngeal carcinoma patients was tested for a precipitating antibody to antigen prepared from Burkitt's lymphoma cells. There is a high incidence (85%) of positive reactions in these patients.

4. A study of the floating population in Hong Kong showed no evidence that nasopharyngeal carcinoma is vector-borne in this high-risk population.

ACKNOWLEDGMENT

I would like to express my grateful thanks to Dr Robert J. Huebner, Chief, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Md., and Dr Herbert F. Oettgen of the Sloan-Kettering Institute for Cancer Research, not only for their help in making this work possible, but also for doing all the serological investigations free. I am indebted to Dr S. D. Stu[illegible]ton, my co-worker in this research, for his encouragement and contributions of material used in this paper. I wish to thank Dr John Grant for advice on the preparation of the manuscript.

TABLE 6

$p = 0.001$

Year	No. of case: All Chinese	Boat people	Others
1956	95	4	91
1957	160	12	148
1958	252	4	253
1959	201	12	219
1960	294	10	284
1961	360	19	343
Total	1 433	61	1,372
1961 population	3 079,901	81 649	2,998,252
Cases/million/annum	8	124	6

nomic standard was conducted There is no evidence to suggest that the high incidence rate is due to any difference in the environment nor is there increased hazard in their environment It is a matter of conjecture that intermarriage might be a genetic factor since it is postulated that inherited traits may determine a degree of susceptibility

Malaria is a notifiable disease, now successfully controlled Sporadic cases occur in both rural and urban areas The immediate environment of the boat population does not favour the breeding of mosquitoes Yellow fever is unknown There is no evidence to suggest or support the hypothesis of a possible vector borne viral aetiology for nasopharyngeal carcinoma in this community

Four histologically confirmed cases of Burkitt s lymphoma have been recorded in Hong Kong none came from the floating population but they were born in Hong Kong and have never left the colony

The unconfirmed hypothesis that Burkitt s lymphoma has a viral cause has led to the possibility that nasopharyngeal carcinoma might be similarly implicated by a virus Since Burkitt s lymphoma is a variant of lymphosarcoma and reticulo-cell sarcoma, and this diagnosis is often confused with nasopharyngeal carcinoma, one might ask if nasopharyngeal carcinoma could be related to Burkitt s lymphoma In Hong Kong almost all cases are carcinoma and the analogy does not suggest itself

To say that there is no evidence that nasopharyngeal carcinoma has any relationship to Burkitt s lymphoma does not of course exclude the possibility of a viral cause for nasopharyngeal carcinoma, which remain a possibility It is again possible that a predisposing virus infection might well be one causing slight or no symptoms. In Hong Kong reovirus has not been isolated up to the present

L. B W Jongkees Mr Laing i probably the best connoisseur and has seen more patients suffering from rhinopharyngeal carcinoma than ybody else. In H lland and in Indonesia cases are seen, but the greatest percentage is in Chinese (pure and mi ed) people Might it be possible that a transport tl n of virus from the mother takes place? We know from the st dies of Bit ers that this takes place in mice with breast cancer (the so called milk factor)

J McA lliffe Curtin In M Laing' large cll ical material of this particular problem, could be say what is the rati of males to females? Also, what is the prognosi i relation t the onset f symptoms with regard to the parent s age?

D Laing (Reply) to Mr *Mi hlke* Your question whether there is f milial tendency t thi disease i a most interesti g one. In my series of er 1600 cases, there were many incidences f two members of a family suffering from thi disease, d even i n f mil there were three members ffliceted The findi g in o e family is highly suggesti e of familial tendency There were seven ff spring, four females and three males. The mother died of nat ral causes, the father of nasophary geal carcinoma, two males d two females died f the same disease two other females both ha ing hist logically confirmed N P C. are still alive and one male is free from this disease

Tw or three members of f mily h ing malignant eoplasms of different organ are not uncommon, d these re most lik ly coin idental, but seven members of one family hav neoplasm in identical anatomical sites, which makes the coincidental planation most u hkely It i possible that inherited traits may determi e a degree f susceptibility to this disease.

T Mr *Jongkees* I will deal with your first quest on f racial susceptibility that Indonesians having Ch ese blood in thei ancestry also ha e high incidence t this disease. This is true not o ly i Indonesia, but also in Th ila d Vietnam, Burma, and th Phillipi es, but i Japa this disease is relatively uncommon, and the in dence rate i comparable t that of the western countries. Similarly the Indians a d Pakistani f Caucasian stock have low i cidence rate.

Your suggestion of a carcinogen c milk factor ilk th t f Bitners, in mice in nasopharyngeal carcinoma, a most i teresti g ne. I must admit I have not thought f this pproach, and I m sure it is worth f llowi g up.

T Mr *M Auliffe Curtin* Th third questi concerned the male and female ratio in this disease I my series it was 2.53 1

Whilst discussi g etiology I w sh to add that the 30–39 g group con tituted about 80% th 40–49 age group being most common making up about 34% I there y relati ship betwee st ge f the disease and prognosi ? Early di g nosis of this disease before th appearance f cervical lymph nodes or euro-opthalmi sympt ms, i the key t good prognosis.

In stage 1 d stage 2 cases, the fi e- ear survival rate i ea ly 40% Early diagnosis is based recog ition f th nasopharyngeal and tologic syndrome whi h I described bout 15 years ago. The first ign of thi is the repeated ppeara ce of blood-streaked spirated post asal secretions, combined with unilateral conductive deaf ess. N sal bstruction i not an ea ly symptom.

Finally a southern Chinese ha i g either the nasopharyngeal or ot logic manifest tion f thi syndrome is assumed t have nasopharyngeal carci oma unt l proved otherwise

ZUSAMMENFASSUNG

Versuche Gewebekulturen von Primärgeschwülsten von 10 Patienten mit nasopharyngealem Karzinom zum Weiterwachsen zu bringen, missglückten. Untersuchungen an chinesischen Patienten mit nasopharyngealem Karzinom zeigten ein relativ höheres Auftreten von Infektionen durch Adenovirus Typ 7 und 12 als die normale chinesische Population. Des weiteren wurden die Seren von 27 chinesischen Patienten mit nasopharyngealem Karzinom auf die Bildung von Antikörpern gegen Antigenpräparate aus Burkitt s Lymphoma-Zellen getestet. Das Testresultat zeigte bei diesen Patienten eine sehr hohe Quote von positiven Reaktionen (85) Im Zusammenhang damit wird die Möglichkeit der Verursachung von nasopharyngealen Karzinomen durch Virus oder in Verbindung mit Burkitt s Lymphoma diskutiert Eine derartige Annahme lässt sich aus den Befunden nicht stützen doch kann die Möglichkeit einer prädisponierenden Wirkung einer Virusinfektion als Initiator maligner Prozesse oder Transformationen nicht ausgeschlossen werden

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50 Gl cest r Buildings, H ng K ng

DISCUSSION

A Miehlke Is there any family predisposition to this disease? Does it exist or not in the Chinese population?

TABLE 1. *ATPase activities in various cochlear structures*

Means with s.e.

Structure	Na-K-ATPase: Moles/kg dr wt/hr	Na-K-ATPase: % of total ATPase	Mg-ATPase (moles/kg dr wt/hr)	Mean activity of turn nos.
Tegm. vasculosum	5.10 ± 0.96	39 ± 3	3.10 ± 1.10	—
Stria vascularis	.93 ± 0.43	59 ± 1	5.57 ± 0.41	1, 2, 3
Lig. spirale				
(a) Behind stria vasc.	0.40 ± 0.09	53 ± 7	0.34 ± 0.09	2, 3
(b) Comprising prom. spir. and sulcus externus	1.60 ± 0.39	56 ± 6	1.26 ± 0.43	2, 3
(c) Bordering scala tymp.	0.35 ± 0.07	36 ± 7	0.62 ± 0.07	2, 3
Reissner's membrane	0.35 ± 0.06	28 ± 4	0.91 ± 0.10	1, 2, 3, 4
Organ of Corti	0.47 ± 0.27	11 ± 6	2.70 ± 0.70	2, 3

by incubation in media, prepared with buffers in the pH range from 6.2–9.6.

The role of Na K–ATPase in the generation of the cochlear potentials (cochlear microphonic potential and +endolymphatic potential) was determined by continuously perfusing the scala vestibuli of the guinea pig cochlea with Krebs Ringer solution during 45 minutes (10 μl/min) with and without ouabain (10^{-}–10^{-4} M). In Table 1 the distribution of the Na–K activated and Mg-activated ATPase activities in the cochlea is demonstrated. The stria vascularis and its homologous structure in the chicken, the tegmentum vasculosum, revealed a very high Na–K–ATPase activity. The Na–K–ATPase activity of Reissner's membrane, organ of Corti, and ligamentum spirale, except part *b* of this structure comprising prominentia spiralis and sulcus externus, is very low. Table 2 gives the activity of the stria vascularis in various turns. There is a decrease for both ATPase activities from the basal to the apical turn; this has also been demonstrated for

TABLE 2. *ATPase activity in various cochlear turns*

Means with s.e.

Turn no.	Na-K-ATPase: Moles/kg dr wt/hr	Na-K-ATPase: % of total ATPase	Mg-ATPase (moles/kg dr wt/hr)	Total dry weight of stria vasc., μg
1	6.37 ± 0.40	56 ± 3	5.01 ± 0.46	14
2	7.03 ± 0.87	61 ± 4	4.32 ± 0.35	12
3	4.60 ± 0.72	57 ± 6	3.40 ± 0.59	7
4	4.35 ± 0.55	58 ± 1	3.14 ± 0.30	3

Acta oto-laryngologica 67 200–205 (1969)

CATION TRANSPORT AND COCHLEAR FUNCTION

W. KUIJPERS

From the Department of Otolaryngology, University of Nijmegen, Nijmegen, Netherlands

Na–K activated ATPase was determined in the cochlear structures by ultramicro-analytical methods. The highest activity was found in the stria vascularis, *with a decrease from base to apex*. Ouabain, which inhibits the isolated enzyme system, appeared to diminish the CMP and +EP to the same extent. These findings strongly suggest that the Na–K ATPase enzyme system in the stria vascularis plays the key role in the maintenance of the cochlear cationic gradients, while such a role appears highly unlikely for Reissner's membrane. The CMP and +EP appear to depend on the functioning of this pump system.

As shown by many investigators, there is a characteristic difference between the ionic composition of the cochlear fluids (Fernández, 1967). The ionic composition of the perilymph is similar to that of other extracellular fluids. The endolymph—being an extracellular fluid—has an ionic composition similar to that of intracellular fluids.

It is now generally accepted that the maintenance of those cationic gradients in most animal cells depends on the activity of a Na–K-activated ATPase that is specifically inhibited by cardiac glycosides (Skou, 1964).

In view of the cationic gradients in the cochlea between endo- and perilymph we have tried to determine whether these gradients are maintained by a Na–K activated ATPase system, both by electrophysiological and ultramicro-enzyme assay techniques.

Enzyme assays were carried out on the cochlear structures of the chicken and the guinea pig. After decapitation the cochlear structures were dissected frozen on dry ice, lyophilized and stored at -25 °C. The frozen-dried structures were weighed on a Kahn electrobalance or on a quartz fiber balance (Lowry, 1953) and incubated in the substrate media as described by Bonting *et al.* (1963). The tissue concentrations varied between 0.2 μg and 2 μg/10 μl medium. Medium A (complete) gave total ATPase activity. The average activity of medium B (no K^+), medium C (no Na^+), medium D (medium A + 10^{-4} *M* ouabain) and medium E (medium B + 10^{-4} *M* ouabain) gave Mg–ATPase activity. Total ATPase activity minus Mg–ATPase activity gave the Na–K–ATPase activity. We also looked at the effect of K^+, Na^+, Mg^{2+} and ouabain *on the Na–K ATPase activity by adding graded amounts* of these substances to the media. The pH activity curves were obtained

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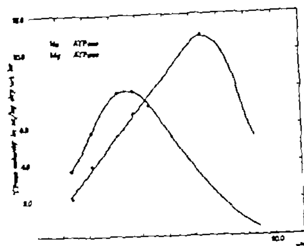


FIG. 2. Effect of pH on Na-K ATPase (O——O) and Mg-ATPase (●——●) activities.

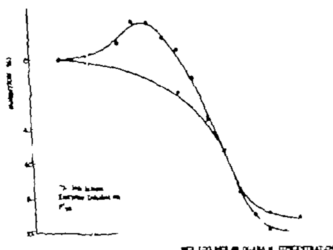


FIG. 3. Effect of ouabain on Na-K-ATPase activity (O——O) and on cochlear microphonic potential (●——●). pI_{50} negative logarithm of the half maximal inhibition concentration.

of ouabain on the isolated enzyme system, also demonstrated in several other tissues (Bonting, 1966) could not be substantiated for CMP.

In conclusion, it can be stated that the inner ear structures possess a Na-K-ATPase system that is similar to those described for other tissues. In view of its high Na-K-ATPase activity the stria vascularis and the tegmentum vasculosum are most likely the sites of the cation pump, which regulates the composition of the endolymph. The role of the other cochlear structures in maintaining the cation gradients between endo- and peri

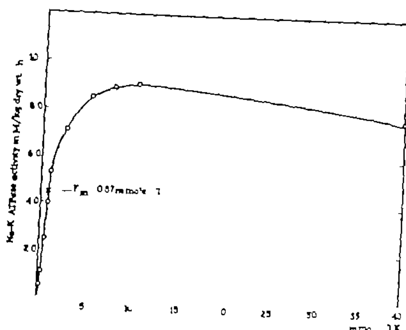


Fig. 1 Effect of K concentration on Na K-ATPase activity

oxygen consumption (Meyer zum Gottesberge *et al.*, 1965) and for LDH and MDH (Kürschner 1968) Comparing the mean enzyme activity for the 1st, 2nd and 3rd turn of the totally dissected stria vascularis (Table 1) with that given in Table 2 for the same separate dissected turns, there is a difference of +22% taking into account the weight of the stria vascularis in these turns. Probably this is due to the time of dissection, which was for the whole cochlear stria quite shorter than for the stria vascularis of the separate turns The decrease in activity from the 1st to the 4th turn cannot be influenced by this, because the apical turns were always first dissected The activities for Reissner's membrane and Organ of Corti should also not be influenced by this effect, because these structures were dissected from cochleae that were lyophilised immediately after decapitation.

The activation of the Na-K-ATPase activity by K is given in Fig 1 Maximal activity was reached at 10 mM K half maximal activation occurring at 0.87 mM With Na maximal Na K-ATPase activity was reached at 10 mM Na and half maximal activation occurred at 4 5 mM Maximal Na-K-ATPase activity was reached with 1-2 mM Mg^{2+} in the medium in the presence of 2 mM ATP The pH activity curves are given in Fig 2 The optimum for Na-K-ATPase activity was pH 7 3 and for Mg-ATPase pH 8.7 Fig 3 represents the ouabain inhibition curve of the isolated enzyme system (open circles) The negative logarithm of the half maximal inhibition concentration was $pI_{50} = 5.5$ The effect of 45 minutes ouabain perfusion of the scala vestibuli on the CMP is also given in Fig 3 (filled circles) Comparing these two inhibition curves, there is a striking similarity in the effect of ouabain on the CMP and the isolated enzyme for both $pI_{50} = 5\ 5$ A similar effect was found for the +EP The stimulating effect of low concentrations

endolymphatic walls of the first turn f the guine pig cochlea. *J Acoust Soc Am* 40 1393.
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DISCUSSION

Ch. von Ilberg (1) Die höchst Aktivität von Na-k-akti ierter ATP se in Sa getiergeweben ist in Erythrozyten Nerven und Gehirnzellen gemessen. Sie ist kein sicherer Indikator für Sekretion, sondern für einen kti en Transport durch Zellmembranen (2) U sere eigenen Potentialmessungen (Ilberg u. Imamura Arch. Exp Ohren Nas. K hlk pf 1966) igen durch Potentialmessu gen an der isolierten Reissner Membran einen akti en Transport durch die Membran.

J Angell-James It is surprising that the ATP se ctivity i Reissner' membran is so low compared with that the stria ascularis. Hughes & Chou measured th metaboli acti ity in several cochlear tissues by estimating the oxy gen uptake.

They found a very high uptake i Reissner' membrane as well as in the stria vascularis, although the former was less than the latter The activity was greater tha that in renal tubules. W th such a large differential in the electrolytes Na and K on th two sides f Reissner' membra e, which is composed f very few cells, we should expect a great metabolic activity to maintain this diff rential.

It is possible that the low ATPase cti ity observed was due to the ery rapid post mortem ha ges which are known to tak place. Th specimens were taken after death and not unde anaesthesia in the li i g a imal.

Hughes & Chou showed th t mixing f endolymph nd perilymph due t en zyme nactivation takes place withi half an hou after death.

W K ljpers (Reply) t *M Ilberg* As far as I know the highest Na-K ATPase cti ty in mammalian cells is found i the kidney The erythrocytes have a very low Na-K ATP se activity The potential that you have measured on the isolated Reissner' membrane need not necessarily be the effect of an active transport mechanism. It may be the effect f passive diffusion.

T Mr *Angell-James* At the congress on cochlear physiology in Halle this year Professor Rauch reported th t ew investigations by him n the O_2 consumptio f Rei sner' membran did t confirm Dr Chou earlier published results. The O_2 consumpt n ppeared much lower Co cerni g the loss of enzyme activity of the cochlea structures post mortem, I can say that the Na-K ATI ase i ery stable enzyme system. In our xperiments o significant loss of enzyme activity was found, keepi g th isolated cochlear structures for more than 1 hour at 0°C.

lymph can be considered negligible The assumption that transport of K from peri to endolymph would occur through Reissner s membrane (Rauch, 1964) must be highly unlikely in view of the low Na–K–ATPase activity not only on a weight basis, but also on an absolute basis. The ratio of the dry weight of the stria vascularis and Reissner s membrane is approximately 3 1 this gives an Na–K–ATPase ratio of 68 1 We may conclude, therefore, that the contribution of Reissner s membrane to active cation transport is very low *Additional arguments for this are the* avascularity and the very high electrical resistance of this membrane (Johnstone *et al.*, 1966) This suggests that the K transport takes place through the spiral ligament and the stria vascularis

The cochlear potentials, CMP and EP appear strongly dependent on the functioning of the Na–K–ATPase system

RÉSUMÉ

L enzyme Na–K–ATPase a été déterminé par des méthodes ultra-micro-analytiques dans la cochlée du cobaye La plus grande activité de cet enzyme a été trouvée dans la strie vasculaire avec une diminution de l activité de la base jusqu'à l apex L ouabaïne qui a un effet inhibiteur sur l enzyme isolé porte le même effet sur le potentiel microphonique et le +EP Ces observations portent à croire que le gradient de cations dans la cochlée formé par le Na–K–ATPase est nécessaire à la fonction cochléaire

ZUSAMMENFASSUNG

In den Innenohrgeweben wird mit ultramikroanalytischen Methoden eine *Ouabain-empfindliche Na-K-aktivierte ATPase nachgewiesen* Die Aktivität war am höchsten in der Stria vascularis, mit einem Abfall von basal nach apikal. Die Potentiale CMP und +EP wurden mit Ouabain in gleichem Masse gehemmt wie das isolierte Enzym Aus diesen Ergebnissen hat sich erwiesen dass die Na-K aktivierte ATPase in der Stria vascularis die zentrale Rolle spielt zur Aufrechthaltung der Kationengradienten im Innenohr dass die Rolle der Reissner Membran hierbei sehr gering ist und dass die CMP und +EP dem Funktionieren der Na–K–ATPase Ionenpumpe abhängig sind.

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DISCUSSION

Ch. von Ilberg (1) Die höchste Aktivität on Na-k-aktivierter ATPase in Saugetiergeweben ist in Erythrozyten Nerven- und Gehirnzellen gemessen. Si ist kein sicherer I dikator für Sekretion, sondern für einen akti en Transport d rch Zellmembranen. (2) U sere igenen Potentialmessungen (Ilberg u Imamura Arch. Exp. Ohren Nas. Kehlkopf 1960) zeigen dur h Potentialmessungen an der isolierten R issner Membran einen akt en Transport durch die Membran.

J Angell James It is surprising that the ATPase acti ity in Rei sner membrane s so low compared with that in the stria ascularis. Hughes & Chou measured the metabolic ctivity in several cochlear tissues by estimati g the xy gen uptake

They found ery high uptak I Reissner's membrane as well as in the stri vascularis, although the former was less than the latter The acti ity wa greater than that i renal tubules. With such large differential in the electrolytes Na and K n the two sides f Reissner's membrane, whi h i composed of very few cells, we should expect great metabolic activity to maintain this differential.

It is possible that the low ATPase activity observed wa due t the very rapid post mortem changes whi h are known to tak place Th specimen were taken after death and not under anaesthesia in the li ing animal.

Hughes & Chou showed that mi ing f ndolymph nd perilymph due to enzyme act t on takes place wit in half an hour after death.

W K lpers (Reply) to Mr *Ilberg* As fa as I know the highest Na-K ATPase activity is mammalian cell is found in the kid ey The erythrocytes ha e a ery low Na-K-ATPase activity The potential that you ha measured on the isolated Reissner's membrane need ot ecessarily be the effect of n active transport mechanism. It may be the effect of passi e d ffusion.

T Mr Angell James At th congress on cochlear physiology in Halle this year Professor Rauch reported that ew in estigatio s by him on the O_2 consumpti n of Reissner's membrane d d t confirm Dr Chou's earlier published results. The O_2 consumption appeared much lower Concerning the loss of enzyme ctivity of the cochlear structures post mortem, I can say that the Na-K ATPase is a very stable enzyme system. I our experiments no significant loss of enzyme activity w s found, keeping the isolated cochlear structures for more tha 1 hour at 0°C.

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ISOENZYMES OF LACTIC DEHYDROGENASE OF THE PERILYMPH AND THE ENDOLYMPH

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Enzyme dehydrogenase is directly related to carbohydrate metabolism. By histochemical methods LDH was found in some hair cells and the stria vascularis. Microchemical methods provided LDH iso-enzyme patterns in the endo- and perilymph

Lactic dehydrogenase is a cytoplasmic enzyme which can be found in all human tissues By histochemical methods, Spoendlin & Balogh (1963) have demonstrated that this enzyme is fairly concentrated in the sensory cells of the Corti s organ of the cat Rauch (1964) has also found it in the vascular stria of the guinea pig Lactic dehydrogenase catalyzes the reversible reaction lactic acid pyruvic acid This fact is important in the intermediary metabolism of carbohydrates.

Lactic dehydrogenase like several other enzymes, can be present under different molecular forms which were named "isoenzymes" in 1959 Their negative electric charges are different and because of this fact they can be separated by electrophoresis LDH1 has the most negative charge, LDH5 has practically no charge LDH1 is prevalent in the oxidation of tissues where aerobic glycolysis dominates on the other hand LDH5 is found particularly in tissues rich in lactic acid and anaerobic glycolysis.

Wieme (1959) showed not only that human tissues, but also rat and mouse tissues, contained the five fractions of LDH and that these fractions were also found in the serum Since then, the dosage of these different fractions has influenced the diagnosis of certain illnesses such as infarctus, hepatic degeneration etc each illness giving a different distribution of the various isoenzymes

Silverstein & Schuknecht (1966) dosed total LDH of the perilymph in cases of otosclerosis, Menière s disease and acoustic tumours. They gave their results in international units. The total LDH value does not seem to produce any significant elements in the diagnosis of these diseases.

More recently Palva & Raunio (1967) have studied isoenzymes of LDH on the endolymph and perilymph of cadaver ears. Non-contaminated endolymph shows, in particular fractions 1 2 and 3 whereas in the perilymph fractions 4 and 5 are also observed These two authors draw particular attention to contamination being a reason for errors

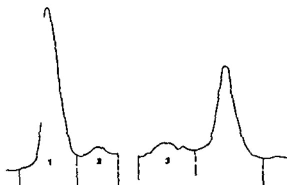


Fig. 1 *LDH isoe zymes of th guinea pigs serum separated by agar-gel lectropho esis*

Our first study of LDH isoenzymes was performed on endolymph and perilymph and compared to the LDH isoenzymes of serum in the guinea pig.

All contaminated samples were excluded By means of marked capillaries, it was possible for us to examine the same quantity of liquid, i.e. 1 or 2 λ This quantity was sufficient to make a differential dosage of LDH on agar gel electrophoresis according to Wieme (1959) It seems superfluous to review this technique because of the full description given by the author

The results obtained by electrophoresis of the serum in five guinea pigs showed that the fractions or isoenzymes of the LDH remained constant. The fraction LDH5 is too insignificant to be taken into account (Fig 1) We would also like to draw attention to a second fact, that there was no important variation, which is also a point in favour of this method (Table 1) and confirms previous data.

We took samples of perilymph and endolymph in the same number of guinea pigs. Tables 2 and 3 summarize th results obtained The important difference in the results for serum on the one hand and perilymph and endolymph on the other lies in the absence of an LDH4 fraction in the two latter liquids. If it existed, it was too minute to be shown.

Table 1 *The LDH isoen-ym s in serum of guinea pigs*

Guinea pig no.	LDH1, %	LDH2, %	LDH3, %	LDH4 %
49	41.4	8.3	10.9	41.2
34	41.5	10.2	15.7	32.7
22	21.8	6.4	32.0	40.8
21	42.1	20.7	17.6	18.6
33	49.4	14.1	6.4	30.9
Average	39.3	11.6	11.8	32.7

TABLE 2 *The LDH isoenzymes in perilymph of guinea pigs*

Guinea pig no	Perilymph LDH1 %	LDH2 %	LDH3 %
40	55.1	21 8	22.9
24	48.3	24 0	27.5
22	50.3	20 9	22.8
31	52.5	20.2	27.2
33	47.9	26.6	25.4
Average	52.4	22.7	25.16

The fact that no LDH4 fraction appeared in the perilymph and endolymph proves that aerobic glycolysis is the only important element for the metabolism of the inner ear This fact is also proved by the much higher content of LDH1 and LDH2 in the perilymph and endolymph In fact, we found 52.5% values in the endolymph and perilymph as opposed to 39% in the serum The LDH2 content is double that of the serum It is also important to draw attention to the fact that the isoenzymes are divided absolutely equally in the same concentration in the perilymph and the endolymph

In the second part of our research, we have been able to show that the distribution of isoenzymes of LDH in humans with normal endolymph and perilymph is comparable to data traced in the guinea pigs As we found in the guinea pig there is no LDH4 fraction in the normal perilymph and endolymph (Table 4)

We took samples of peri and endolymph in several cases of pathology of the inner ear or the ponto-cerebellar angle. Without venturing on a definite conclusion because of the small number of cases, we feel justified in drawing attention to certain significant facts taken from a diagnostic

TABLE 3 *The LDH isoenzymes in endolymph of guinea pigs*

Guinea pig no.	Endolymph LDH1 %	LDH2 %	LDH3
49	48 9	23.4	27 8
4	53.2	23 1	23.7
22	53.2	21 7	22.1
31	59 7	23.3	16.9
33	51 0	24 0	25.0
Average	53.2	23.6	23.1

TABLE 4. *Comparison between the different curves of isoenzymes of the serum of the perilymph and of the endolymph*

	LDH1 %	LDH2, %	LDH3, %	LDH4 %
Human serum	47.4	33.1	13.1	6.4
Human perilymph	40.5	22.2	37.2	—
Human endolymph	39.2	36.2	22.8	—

TABLE 5. *The LDH isoenzymes in normal human perilymph and in pathological conditions*

	LDH1 %	LDH2, %	LDH3, %	LDH4 %
Normal human perilymph	40.5	22.2	37.2	—
Meningioma of the fossa posterior	33.4	27.0	18.6	20.8
Menière's disease	40.5	23.0	36.0	—
Cholesteatoma of the fossa posterior	33.7	33.1	19.1	13.8
Cerebellar tumour (astrocytoma)	43.1	37.7	19.1	—

point of view. These are given in Table 5. From this table it appears that in some cases of tumours in connection with perilymph circulation the LDH4 fraction is present.

These results justify a continuation of our research. In fact, there is clear evidence of the fraction LDH4 in certain tumours of the ponto-cerebellar angle. Perhaps if we continue our research it will be possible to use this new means for differential diagnosis between Menière's disease and tumours of the ponto-cerebellar angle.

RESUME

Lenzyme L.D.H. a un rôle importa t dans le métabolisme des hydrates de carbon. L'examen histologique a démontre sa présence au niveau de la stri vasculaire et des cellules sensorielles. Grâce aux techniques microchimiques, on peut doser L.D.H d l endo- et de la perilymphe. Nous vous pu fractionner les isoenzymes de la L.D.H en utilisant les techniques de fractionnement des protéines.

ZUSAMMENFASSUNG

Die Dehydrogenase ist wesentlich für den Karbohydratmetabolismus. Mit Hilfe histochemischer M thoden wurd L.D.H. in Haarzelle und Stria-V scularis da gestellt mit mikrochemischen- und analytischen Meßverfahren konnten wir die Isoenzymes der L.D.H. in Endo- und Perilymphe dosieren.

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DISCUSSION

T Palva I was very much interested in Mr Kluyskens's paper because I have been working in the same field for a couple of years. His conclusions bring immediately to mind the studies of Silverstein & Schuknecht who found high perilymph total protein concentrations (average over 2100 mg%) in cases of acoustic neuromas. It may very well be that in future the use of microchemical methods will greatly increase our diagnostic possibilities, since taking perilymph samples is quite easy through the stapes footplate LDH could very well be used in this respect too since we know that it appears also in cerebral extract, and that the cochlear aqueduct is capable of letting pass various substances from the cerebrospinal fluid space into the perilymph

We have been able to show also small fractions of LDH and LDH_5 in the perilymph This may be due to the fact that we have been using agarose and not agar which gives a poorer resolution of these isoenzymes. This was pointed out by Wieme in 1966 and was confirmed in our studies.

Another factor that also has a bearing on LDH and LDH_5 findings is the storing of the fluids before the analysis is due These isoenzymes may disappear if analyses are not made soon after the drawing of the specimens and I should like to know if there was any greater time delay before performance of the electrophoresis. Lastly the contamination of the samples is our present problem. The contamination with red cells which lack LDH_5 is for this fraction negligible but a possibility with LDH Has Mr Kluyskens any idea how big a factor this might have been in his pathological cases?

P Kluyskens (Reply) to Mr *Palva* I do agree that the origin of the modification of the isoenzym pattern may be located in the central nervous system. It has been demonstrated that the LDH and LDH_5 fractions are more evident when agarase is used. We only used the agar-gel electrophoresis on unstored endo- or perilymph.

In many cases the contamination by blood cells or serum cannot be avoided, but it is less when an electrocoagulation of the mucous membrane takes place before a small hole is bored in the footplate.

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THE TOTAL PROTEIN OF HUMAN PERILYMPH

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Total protein values were determined from the perilymph of 47 cadaver ears. After subtraction f hemoglobi th average t tal prot in for two series of 27 and 10 ears was 553 a d 559 mg% respecti ly When the perilymph specimen were taken directly without freezi g the verage total protei f gures were reduced to 280 (cochlea) or 312 mg% (vesti bulum) The a erage hemoglobin concentrat in the latter series was 42 and 39 mg% as compared with th values 124 and 118 mg% in the former two series. Albumi was th mai constituent of perilymph prot ins, accou ting for more than half of the total. It is co cluded that values representing the living normal st te in man re difficult to b-tain but, allowing for th post-mortem changes in CSF total protein, the true alues may be estimated at 50 t 100 mg%

Only a few analyses of the protein content in human perilymph have been made so far and the data reported are not uniform. Obviously this is due to the difficulty of obtaining human perilymph specimens during life without injuring the hearing and to the fact that the purity of the specimens leaves much to be desired

In 1950 Wallner & Raymond reported on five cases of Menière s disease in which they compared the values for perilymph from the horizontal semi circular canal with those for the cerebrospinal fluid (CSF) using the ultra-violet absorption test. In conformity with visual estimation of perilymph, there was a strong peak at 410 millimicrons, testifying to the presence of hemoglobin another peak was observed at 275 mmicrons, end-absorption appearing at 265 Analyses of spinal fluid showed an absence of hemoglobin low bsorption t 275 mmicron with an endpoint t 265 Comparison of the absorption curves suggested that the protein content of perilymph is more than twice that of CSF

In 1938, R uch & Köstlin, without giving any details of their procedure reported that the total protein content in human (post mort m) perilymph and endolymph is 70–100 mg% and 20–30 mg% respectively

Hladky *et al* (1960) obtained 26 perilymph specimens in otosclerosis and ne in Menière s disease reporting total prot in values varying from 115 to 350 mg% The samples were contaminated with blood up to 10% centri-

This study as supported by grant from the N tional Research Council for Medical Sciences.

fuge separation of erythrocytes generally yielded values close to the lower limit of the range given

Recently Schindler *et al* (1965) recorded an average value of 728±463 mg% for 13 perilymph samples, mainly from otosclerotic ears, with blood contamination of less than 3 5% The amount of protein deriving from blood was estimated at 200 mg% which results in an average value 528 mg% In a series reported by Rüedi *et al.*, consisting of 30 otosclerotic ears, the total protein value was 965±430 mg% From this value the possible effect of mixed blood had already been subtracted.

Silverstein & Schuknecht (1966) reported the total protein as ranging from 150 to 770 mg% (mean 425) in 12 otosclerotic ears In eight further cases with poor bone conduction the range was 130–825 mg% (mean 380 mg%) Similar values were found in nine cases of Menière s disease (range from 140 to 608 mg% mean 348) Widely differing results were found in seven cases of acoustic tumor here the range was 1400–3600 mg% and the mean as high as 2444 In a later series of 12 otosclerotic ears, the use of a meticulous technique to avoid blood contamination gave an average concentration of 195 mg% with a range of 89 to 326 In 10 normal ears studied $1^1/_2$ to $7^1/_2$ hours post mortem the average was 212 mg% (range 135–509)

Further results from Schuknecht s clinic were reported by Davies (1968) Within the limits of 1 to 24 hours post mortem there was no appreciable difference in the total protein figures, and a mean of 282 mg% (range 49–509) was obtained

MATERIAL AND METHODS

Perilymph from 47 ears was collected post mortem using the methods described earlier (Palva & Raunio, 1967) After death the bodies were kept at +5 C until autopsied the removed perilymph specimens were kept in a deep freezer until analyzed Measurement of the total protein was performed by the method of Lowry *et al* (1951) An immunochemical method was employed for albumin determination (Mancini *et al.*, 1965) Hemoglobin content was determined on the basis of peroxidase activity of hemoglobin as suggested by Crosby & Furth (1956)

RESULTS

The first series of analyses were made on perilymph from 27 cadaver ears, generally removed 36 to 48 hours after death The temporal bones were stored in a −40 C deep freezer until there were several pairs. Perilymph samples were taken after extraction of the stapes as soon as the cochlear fluids were defrozen at room temperature The results are seen in Table 1 The total protein value was found to average 678 mg% whereas the average hemoglobin concentration was 124 mg% When the individual hemoglobin values were subtracted from the initial total protein values, the figures shown in the third column were obtained The final average was 554 mg%

On reviewing these rather high results, it was decided to analyze a second

TABLE 1 *Perilymph protein analyses from 27 frozen specimens*

	Total protein	Hb	Total protein (Hb subtracted)
Mean	678	124	554
Range	460–1194	18–303	393–1076
S.D.	182	73	162

series in which the corresponding CSF values were included. The results for 10 such ears and CSF specimens are presented in Table 2. The perilymph analyses still yielded values similar to those in Table 1 (average 559 mg%) but the figures for CSF were all (with one exception) under 200 mg%. The extreme CSF value, 1166 mg% was found in a 58-year-old man who had died of coronary occlusion without signs of neurological disease. In this case as in all others, hemoglobin contamination of the CSF was very slight.

Following the discovery of this fairly great discrepancy between perilymph and CSF protein values, 10 further temporal bones were removed and perilymph collected immediately. Samples were taken first through the round window membrane and then from the vestibular labyrinth. The albumin content of each sample was also determined.

The results in Table 3 show that, after subtraction of the hemoglobin values, the remaining total protein from the scala tympani averaged 280 mg% and that from the vestibule 312 mg%. These values are not statistically different but both differ highly significantly from the values presented in Tables 1 and 2 ($p < 0.0025$). The average albumin content of the perilymph

TABLE 2 *Perilymph and CSF protein analyses from frozen specimens*

	Perilymph			CSF		
Case no.	Total protein	Hb	Total protein (Hb subtracted)	Total protein	Hb	Total protein (Hb subtracted)
1 sin	876	131	745	147	0	147
2. sin	1200	40	1160	181	7	174
3. dx	878	295	583	150	0	150
sin	471	55	416			
4. sin	876	255	621	177	0	177
dx	389	51	338			
5 sin	715	113	602	1166	4	1161
dx	533	63	470			
6. dx	358	64	294	187	0	187
sin	478	117	361			
Mean	677	118	559	334	2	333
Range	358–1200	40–295	338–1160	147–1166	0–7	147–1161

TABLE 3 *Protein analyses on non fro en cochlear and vestibular perilymph*

Case no	Cochlear perilymph: Total protein	Hb	Total protein (Hb subtracted)	Alb	Vestibular perilymph: Total protein	Hb	Total protein (Hb subtracted)	Alb	CSF: Total protein	Hb	Total protein (Hb subtracted)	Alb
1 *dx*	203	7	286	110	300	2	298	110	203	0	203	[illegible]
sin	16	3	243	110	293	0	293	111				
2. *dx*	328	5	323	—	338	4	334	250	680	4	676	11[illegible]
sin	395	48	347	180	309	5	361	250				
3. *dx*	437	135	302	290	521	114	407	300	620	51	569	[illegible]
sin	419	113	306	180	414	48	366	270				
4 *dx*	250	30	220	45	347	65	282	150	139	8	131	[illegible]
sin	297	19	2 8	110	489	145	344	250				
6. *dx*	216	5	211	78	197	8	189	88	149	0	149	[illegible]
sin	330	58	281	96	246	2	244	98				
Mean	322	42	280	133	351	39	312	187	358	13	345	[illegible]
Range	216–437	3–135	211–347	78–290	197–521	0–145	189–407	88–300	139–680	0–51	131–676	[illegible]

samples from both sites accounts roughly for half of the total protein figure The CSF total protein was much elevated in two cases (620 and 680 mg%) both were old people with pronounced atherosclerosis in the basal brain arteries but without any other central nervous system lesions. The hemoglobin was 51 mg% in one case and less than 10 mg% in all others

DISCUSSION

In these post mortem analyses, the factor apparently responsible for the high average values for total protein in Tables 1 and 2 was the process of freezing and defreezing before collection of the fluid specimens In fact, perilymph was removed from two ears in icy crystals and the resulting figures were comparable to those in Table 3 thus storing of the bones as such did not increase the protein content During the defreezing process both hemoglobin and plasma proteins may issue from the damaged capillaries into the perilymph space This is apparent from Tables 1 to 3 which indicate that the hemoglobin concentration of perilymph from non-frozen specimens is only 40% of that in the frozen specimens.

It is interesting to note that our values from frozen specimens are of the same order as those obtained by Schindler *et al* (1965) and clearly lower than those of Rüedi *et al* (1965) This points to the same source of contamination, viz. erythrocytes and plasma which is inevitably present at routine stapedectomies Thus the high figures for total protein in otosclerosis probably are artefact Case 3 included in Table 3, showed otosclerosis in the right ear nevertheless, the value for total protein did not differ from

the other cases in any way This is in conformity with the results of Hladky *et al* (1960) and Silverstein & Schuknecht (1966)

The values for perilymph, taken 24 to 72 hours post mortem (Table 3) coincide fairly well with those of Davies (mean 282 mg% range 49–509 mg%) whose 25 samples were all taken within 24 hours (six within two hours) although the hemoglobin concentration was not analyzed and subtracted. It is worth noticing that time within the limits 1–2 hours or 2–24 hours, had no effect upon the total protein values of Davies, and in our series they remained at the same level as late as 72 hours after death. Apparently the cessation of blood flow was responsible within the first hour for the post-mortem values, and these are not true values for living patients. This result is supported by the findings made by Rodgers & Chou (1966) and ourselves (Palva & Tikanmäki, 1969) concerning post mortem K and Na values, which were not found to change appreciably after the first few post-mortem hours.

Our figures for post mortem CSF total protein generally were below 200 mg% however there were three cases in which the values were much higher without apparent cause The contamination of CSF with hemoglobin is generally low Thus the artificial increase in the total protein content of post-mortem CSF pecimens probably is lower than in perilymph.

Determination of albumin by the immunodiffusion method was carried out because we had earlier reached the conclusion (Palva & Raunio, 1967) that this protein is the main normal constituent of true perilymph proteins. The amount of albumin in the samples of this study (Table 3) concurs with this view

Since both the post-mortem and intra-operation values do not represent the true tatus of human perilymph concentrations, one may truly wonder whether the real figures during life are known t all. As regards CSF there is no doubt the normal values vary between 15 and 30 mg% figures below 45–50 mg% still being n rmal For perilymph, no investigators other than Rauch & Köstlin have claimed to state normal values (between 70–100 mg%) Study of the Rauch-Köstlin (1958) report, however gives no clues as to how th total protein figures were obtained. In Rauch s monography (1964) these figures were stated to be post mortem

Nevertheless, when the gen ral post mortem values of 150–200 mg% for CSF (Table 3) and the true normal figures of less than 50 mg% are con sidered ne cann t avoid the conclusion that a somewhat larger artificial increase to the perilymph alne would reduce the average here obtained, 280–312 mg% to some 50–100 mg% in the normal living state This, how ever remains speculative.

RESUME

Le taux de la protéin totale de la *périlymphe* humaine a été determiné dans 40 cas (post mortem) Les résultat sont influencés par conséquence, par les com-

posants du sérum On a déterminé aussi la protéine totale et l'hémoglobine du liquide céphalo-rachidien ainsi que la concentration d albumine dans les deux liquides.

ZUSAMMENFASSUNG

Die Gesamtproteine wurden von der Perilymphe in 47 Kadaver Ohren bestimmt. Nach der Subtraktion von Hemoglobin Werte waren die Gesamtproteine in zwei Serien von 27 und 10 Ohren 503 und 559 mg% Direkte Proben ohne Gefrierung der Temporalknochen für Bewahrung gaben reduzierte Werte von 230 (Cochlea) oder 312 mg% (Vestibulum) für die Gesamtproteine Die Mittelwerte für Hemoglobin in diesen Proben waren 42 und 39 mg% während die für die gefrorenen Spezimen 124 und 118 mg% waren Albumin machte ungefähr 60% von den Gesamtproteinen aus. Die Schlussfolgerung ist, dass rechte Normalwerte für lebende normalhörige Menschen kaum zu finden sind gestützt bei den Analysen von post mortem Zerebrospinalflüssigkeit, diese sind wahrscheinlich von der Ordnung von 50 bis 100 mg%

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PRESBYCUSIS, PRESBYSTASIS AND PRESBYOSMIA AS CONSEQUENCES OF THE ANALOGOUS BIOLOGICAL PROCESS

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We found i the basal coil of the spiral tractus and in the depth of the internal auditory meatus a reduction in the number of the openi gs through whi h the nerve fibres f the coustic nerve pass. Thi reduction was due t the appositi n of bony substance On the sections through the acoustic nerve of the same subject, the number of f bres wa also reduced in the corresponding rea. Analogous cha ges were found in the regi n of vestibular areas and also the posterior part of the cribriform plat of the ethmoidal bone. These three places are similar in shape, i.e. consisting f a system f small penings through which pass the erve fibres. I all these three places the appositi n of bony substa ces increases with ge and i all these three org ns the fu ction diminishes with age W believe, theref re, that pposition of bone causes compression nd degeneration f erve fibres and hence the senile changes in hearing, vestibular function, and smell.

It is well known that hearing diminishes in old age but it is not so clearly understood that the vestibular and olfactory functions are also reduced. We believe that these three physiological changes which develop progressively with aging are caused by an analogous biological process.

Impairment of hearing in advanced age, known by the name of primary idiopathic presbycusis, is a deafness of the perceptive type, without recruit ment, which means that the changes are not primarily localised in the organ of Corti. It is characterised by a loss of hearing of high tones, so that the changes must be localised in the basal coil of the cochlea. The longest frequences of 250–500 Hz, are preserved, whil frequences of 1000–2000 Hz are impaired much earlier Frequences of 4000–8000 Hz disappear as early as the 40th year

Rossberg (1964) found on clinical material that the excitability of the vestibular system is also reduced with age, but this fact is not often discussed in the literature

The conception of progressive senile anosmia is based on histological changes described by Prévost (1958) but very little attention has been paid to this problem Only few authors (Portmann *et al* 1960 Hofmann, 1926 Vaschide, 1903) mention it.

The reason why the changes on the cochlear apparatus have been investigated and discussed more than those on the vestibular and olfactory appa-

ratus lies in the fact that the changes in hearing become manifest sooner than those of the vestibular and olfactory apparatus. The vestibular apparatus has a greater possibility of compensation. Slight changes in the olfactory apparatus cannot easily be registered either by subjective or objective methods, so that they remain hidden for a long time. In the ear on the other hand the loss of some frequences, especially in musically trained people, is very early registered by the patient himself and objective methods of examination

The cochlear the vestibular nerve and the fila olfactoria belong to sensory nerves and they conduct irritations from the corresponding sensory organs. All these three nerves pass, on their way from the sensory epithelium to the central nervous system through a system of tiny holes in a thin bony plate We found in the region of these tiny holes a progressive piling up of the bony substance from the fetal period to the end of life

Examining a relatively large material of 100 skulls (200 pyramides) the fundus of the internal auditory meatus from the fetal period to 85 years of age under binocular lupe (× 10) we found that the number of holes in the spiral tract as well as in the vestibular areas diminishes progressively with age The reduction of the number of holes was bigger in the basal turn than in the modiolar region On cross-sections through the spiral tractus we could establish that from the fetal period to the adult period the endostal bone which forms the tractus increases in thickness by ten Analogous thickening of the bony substance and reduction of the number of openings in the vestibular areas and saccular and utriculoampullar area were more evident when present because the number of holes, being smaller from the beginning could more easily be counted (Fig 1) This piling up of the bony substance develops progressively

At the end of the sixth or the beginning of the seventh fetal month, the openings in the spiral tractus are big and the bony substance delimiting them very scarce By apposition of the bony substance in the region of the tractus, the holes become smaller and the bony substance between them thicker This apposition is in the beginning more intensive in the region of the basal coil In some cases the piling up of the bony substance is not uniform so that on some places there remain excavations in the bottom of which a certain number of holes for the nerve fibres can be seen In fetuses the number of holes varies from 100–140 and in adults it diminishes by a third As a result of the apposition of bony substance, we found in 13 specimens from the age 64–78 years in the region of the entrance of the cochlear branch of the auditory artery bony cuffs surrounding the artery According to Schwartze the branches of the internal auditory artery enter the cochlea through the central canal and through one of the openings in the spiral tract We found the cuffs in all mentioned cases only in the beginning of the basal coil except in one where there was at the same time also a cuff in the region of the central canal of the modiolus.



Fig. 1



Fig. 2.

Fig. 1 A section through the acoustic nerv I the region f the tract piralis (63-yea old person) One ca see the rarefication of the nerv f bres I the basal coil I comparison ith the modiolar region.

Fig. 2. Reduction of th holes I the estibul area f the f ndus.

The fact that the bony cuffs appear in the greatest number of cases in the basal coil is something that we must expect, because it is the place where the hyperostotic changes first develop, narrowing at the beginning and finally obliterating the holes for the fibres of the acoustic nerve, while the modiolus remains intact for a l ng time

To be able to demonstrate the consequences of these changes in the fundus on the nerve fibres, we made histological sections through the acoustic nerve near the fundus, i.e in the region where it immitates in shape the shape of the spiral tract.

The nerve is, in the region of fundus, divided into small bundles and has an envelope of connective tissue to a length of 2 mm From that point centrally we found glial elements in the nerve and the bundles join to form a single nerve trunk.

On the nerve which we succeeded in cutting through in the region very close to the fundu in older people, where the holes of the tractus are reduced, we have seen that the number of nerve fibres in this region is also reduced (Fig. 2)

We did not count systematically the number of nerve fibres in the acoustic nd vestibular nerve because we could, by the number we hav counted, prove the findings of Rasmu sen (1940) According to Rasmussen the num ber of cochlear fibres is educed with age by more than half compared with the estibular fibres. The absolute number of cochlear fibres is bigger than th t of the vestibular fibres. The reducti n of fibres is in general symmetrical as well as the reduction of the number of openings in the spiral tra t.



FIG. 3 Reduction of the holes in the lamina cribrosa (66 years) compared with the case where the holes were wide and numerous (20 years)

Unfortunately we did not have the possibility of purchasing the audiogramm of the specimens from which we examined the fundus and the nerve because these specimens were obtained during dissections in forensic medicine and pathological anatomy

A completely analogous process was also found in a third region, where the nerve fibres also run through a system of tiny holes, i e in the region of the lamina cribrosa of the etmoid The number of holes is reduced here too in the *postero-anterior* direction by a thickening of the lamina cribrosa. This fact has already been described by *Knajfel* We could prove this reduction on our material We found cases with a complete disappearance of holes and nerve fibres in the posterior region of the cribrous plate It was easier here to prove the reduction in a number of holes because the cribrous plate is more accessible than the fundus and the number of holes is not so big as in the spiral tract (Fig 3)

Summarising all these findings, we came to the conclusion that all these three processes in old age are consequences of the piling up of the bony substance in the region of the nerve openings This bony substance narrows the holes, forming tiny bony channels which are finally obliterated The piling up of bony substance leads to compression of the nerve fibres and consequently to their atrophy followed by a loss of function

We called by analogy with presbycusis, the changes of the vestibular and olfactory function due to aging *presbystasis* and *presbyosmia*

RÉSUMÉ

Nous avons trouvé dans la région du crible spiroïde dans le conduit auditif interne une réduction du nombre des petits canaux par lesquels passent les fibres nerveuses du nerf acoustique. Cette réduction était due à une apposition osseuse. Sur les coupes à travers le nerf acoustique du même individu le nombre des fibres nerveuses était reduit aussi dans la région correspondante. Des changements analogues ont été trouvés aussi dans les régions vestibulaires, ainsi que dans la partie postérieure de la lame criblée de l'ethmoïde. Ces trois places sont très semblables en ce qui concerne le configuration, c est à dire elles possèdent un système de petits canaux pour les fibres nerveuses. Dans ces trois places l'apposition osseuse accroît avec l'âge et la fonction de ces trois organes est réduite avec l'âge. Nous pensons que cette apposition osseuse produit une compression et une dégénerescence des fibres nerveuses et par conséquent des changements seniles de l'ouïe, de l'appareil vestibulaire et de l'odorat.

ZUSAMMENFASSUNG

Wir haben in dem der Basalwindung entsprechenden Teil des Tractus spirali im Boden des inneren Gehörganges eine Verminderung der Zahl der Öffnungen für die Nervenfasern des N. acusticus durch Auflagerung von Knochensubstanz gefunden. An den Schnitten durch den N. acusticus von demselben Individuum konnte man eine Verminderung der Nervenfaserzahl in der entsprechenden Gegend feststellen. Analoge Veränderungen fanden wir in den vestibulären Areen sowie in der hinteren Partie der Lamina cribrosa des Ethmoids. Diese drei Stellen sind ähnlich gebaut d.h. sie enthalten ein System von Öffnungen für den Durchtritt der Nervenfasern. An diesen drei Stellen vermehrt sich die Auflagerung von Knochensubstanz mit dem Alter und alle drei Organe sind durch eine Funktionsherabsetzung im Alter charakterisiert. Wir glauben deswegen, dass die Knochenauflagerung eine Kompression und Degeneration der Nervenfasern verursacht und somit die senilen Störungen des Gehörs, des Gleichgewichts und des Riechorgans.

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DISCUSSION

M Portmann Félicitation pour cette magnifique collection d os temporaux. Cependant je ne suis pas tout à fait d accord sur les conclusions très mécaniques avancées selon lesquelles c est la compression qui est la cause primaire de l'atrophie du nerf L os est le resultat d un équilibre biologique très fin il est probable que l os ferme les espaces parceque le nerf a primitivement dégéneré et non l'inverse.

C S Hallpike Do you think that the bony changes which you have observed might have a pathogenesis similar to those results shown by Mellanby from Vitamin A deprivation?

H H Naumann Frau Krmpotić sprach von der Schwierigkeit, die olfaktorische Leistung zu messen Wir haben an der Berliner Klinik eine Apparatur entwickelt, mit welcher wir 3 Parameter für die Riechfunktion messen können (1) bei Latenzzeit (bis zum Beginn der Riechsensation) (2) die relative Menge des angebotenen Riechstoffs und (3) des Luftvolumen welches als Träger für die Riechsubstanz dient. — Wir haben Messungen an Serien von Versuchspersonen verschiedenen Alters bezuglich des Riechvermögens durchgeführt. Unsere Ergebnisse

bestätigen, dass beim alten Menschen di ben genannten Paramete signifikant schlechtere Ergebnisse geben als beim Jung n.

M v. Eyck Existe-t-il un parallelisme parfait entre le phénomène de fermeture des orif ces osseux et la quantité des cellules sensorielles dégé érées? A ez vous observé des cas où les cellules sont dégénérées alors qu'il n'y a pas d'occlusi n des orif ces osseux?

A Monland n J ai également admiré les beaux documents an t miques de Mme Krmpotić. Nous avons fait une série d examens vestibulométriques qui n ont pas révélé de modification du seuil vestibulaire vraiment significative que l'on puisse comparer à une *presbyacousie* Aussi j'aimerais demander à Mme Krmpotić si elle ne pense pas que l'élément asculaire qu elle constaté joue u ôle plus important d ns la pathogénie des troubles de presbystasis que les altérations osseuses?

H Engström It is very often difficult t state what th primary reason is for a modification f the anat my as described here Could it be, a we know from Bredberg's studies in the ea and Naessen studies i the olfactory regi n, that with increasing age considerable number f sensory cells and nerve fibres—ganglion cells degenerate and for that reason fewer and smaller channels are needed i the bone. Could the narrowing thus be a consequence not the reason.

J Krmpotić (Réponse) à Mr *Portmann* C'est vrai qu'il est difficile quelque fois de dire ce qui est primaire et ce qui est secondaire mais nous vons l'impression d prè nos études sur un grand n mbre des préparati ns de la période fétale jusqu' à l'âge vance que l'apposition osseuse est p imaire et la dégénération osseuce secondaire.

T Mr *H Upike* We have not studied th relationsh p between the hanges f the bone nd a cristanimosis, as y t. W believe that in case of presbyacusis the liquor does not play an important role in th cha ges of the bo e because in the utricular a d vascular regions this contact with the liquor is ot so close and the hanges are analogous as n th cochlear regio

An H *Naumann* Ich gratuliere Hr Naumann dass er die Moglichk t hat di Riechmessungen so exakt durch ufführen wir haber leider   ht di dazu nötigen Geräte Es freut mich aber dass er uch bjekti ein Nachla f des Riechvermögens mit dem Alter feststellen konnte

T M   *Eg k* W are very sorry to say that for technical reason we did not have the possibility to f llow i the same subject the fat   f th nerve cells, nerve f bres, and bon but we are do g our best t find   way to compare the udiogram before death with post-mortem findi g   th bone organ of Corti, a d nerv fibres.

à Mr *Monland n* Le f cteur vasculaire joue sa   d ute ussi un rôle mais selon nos études sur un gra d ombre d os temporaux nou   vons l'impression que l'apposition osseuse est d'une mportance prévalente

T M *Engström* Out of the previou ly menti ed reasons, we ca   ot say what h ppen with th ga glion cells and the hai cells. We did not hav here the opportunity t examin in the same subject the ear and nose, so we cannot say anything about the parallelism of these processes i the same indi idual.

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THE INNERVATION OF THE DEVELOPING FOWL EMBRYO OTOCYST *IN VIVO* AND *IN VITRO*

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The development of the innervation of the sensory neuro-epithelium of the inner ear has been studied in organ cultures of the chicken-embryo otocyst and in the developing chicken embryo with particular reference to the origin of the nerve endings and neurones. It is suggested that they originate from their embryonic centres in the mesenchymatous tissue surrounding the otocyst and reach the neuro-epithelial cells through gaps in the otic capsule and the basement membrane

The innervation of the inner ear has attracted much interest and one may recognise several phases in the progress of the neuro-histology of the inner ear roughly coinciding with the advances in our microscopical and histological techniques Light microscopists such as Cajal Held and others have laid the foundations and erected the building of neuro-histology more recently the electron microscopists have provided the internal decoration. Engstrom Wersäll Spoendlin Catherine Smith and others have played an important pioneering role in this field.

Our studies on which the present paper is based have been carried out on tissue cultures of the isolated fowl embryo otocyst which have shown that the otocyst attains *in vitro* a high degree of differentiation at both the cellular and ultrastructural level although isolated from the central nervous system (Friedmann 1956 1959 1965 1968 Friedmann & Bird 1961 1967)

It is perhaps interesting to note that the technique of tissue culture was, in fact born out of the great controversy between the supporters of the neurone or outgrowth theory among whom Cajal played a prominent role and of the supporters of Hensen s unitarian theory of the development of the nerve fibre

Early in this century Ramon y Cajal s discoveries concerning the embryonic neurone were taken to a further stage by Ross Granville Harrison who in the first decade of this century was the first to demonstrate *in vitro* on a coverslip coated with clotted frog lymph that the nerve fibre developed from a nerve cell and to confirm experimentally the validity of the outgrowth theory (Harrison, 1907 and Hughes, 1968) this was formulated by His, who in a paper on the developing human cord (Hiss, 1880) concluded that every nerve fibre originated from a single cell

The views of Cajal, His and Harrison have by no means commanded a unanimous and final acceptance (Hughes, 1968) Hensen, the great opponent of the outgrowth theory" maintained that the cells of a nerve center and its related end-organ arise from the division of a common parent cell and that the sensory nerve fibre between them originates from an initial connecting filament (Hensen, 1864)

As regards the innervation of the inner ear the outgrowth theory has again been challanged by Proctor & Proctor (1967) in an interesting paper "On the understanding of hereditary deafness The authors claim to have shown in 71-hour fowl embryos "that the cells of the acoustic ganglion do indeed arise from the neuroepithelium of the otic vesicle. By 78 hours, the ganglion cells have formed from the wall of the otic vesicle the innervation of the future sensory hair cells is accomplished At 92 hours in the chick embryo the auditory ganglia migrate away from the auditory vesicle" (Proctor & Proctor 1967) These conclusions appear to be based on light microscopic evidence alone and on the description of a group of cells in the subepithelial tissues of the young otocyst considered to be neurones

It has been our experience that in the early stages of development, the neurones are concentrated in the stato-acoustic ganglion or its anlage" near the neural crest. We have in fact suggested that the explant should include some of these neurogenic elements so as to achieve complete differentiation and innervation of the sensory epithelium (Friedmann, 1956, 1965 1968 Friedmann & Bird, 1961 1967) Scattered neurones may be seen in the differentiated otocyst in the mesenchymatous tissue underneath the epithelium.

The present investigations have had the object further to clarify some of these important issues. Electron microscopic evidence will be presented that the large cell referred to by Proctor & Proctor are Schwann cells and often contain large fascicles of non-myelinated nerve axons which are derived from the neur nes f the stato-acoustic ganglion. All the evidence to be described contradicts the suggestion that the cells of the acoustic ganglion could arise from the neuroepithelium of the otic vesicle and fully endorses the outgrowth theory of Hiss (1968) Ramon y Cajal (1960) and Harrison (1907)

METHODS AND MATERIAL

The watchglass method of Fell & R bison (1929) was used, as modified in our tissue culture laboratory Over 0000 fowl embryo otocysts have been processed in 152 experiments (Friedmann & Bird, 1961 Friedmann, 1968) In th present experiments, 144 otocysts have been studied, and were grown both in air nd in a controlled atmosphere of 9 per cent O_2 and 5 per cent CO in a gla s container Eight ten and twelve-day-old cultures were collected, fixed in 3 per cent buffered glutaraldehyde followed by osmic acid (Zetterqvi t, 1956) and embedded in araldite Sections were cut on an

L K B III Ultrotome an stained with uranyl acetate and with the simplified leadcitrate stain of Venable & Coggeshall (1965) and examined under a Siemens Elmiskop I

Embryo otocysts were obtained from fertilised hen-eggs incubated at 38 C The incubated eggs were successively opened after 1 2, 3 to 12 days incubation the embryos removed and the otocysts dissected and processed as above

Own Observations

The otocyst of the 3 to 5 days fowl embryo, which served as the initial explant for tissue culture is lined by an undifferentiated epithelium which consists of several layers of cells The surface layer is composed of tall cells the basal cells are cuboidal to polygonal in shape and rest on a basement membrane which may be absent in places. The indistinct cell membranes appear to have gaps and the epithelium has a syncitial appearance Fig 1 shows a 5-day-old embryo with similar features. Ribosomes and polyribosomes are abundant in the cytoplasm which contains a smaller number of mitochondria becoming more numerous as the otocyst matures. The Golgi apparatus is less well developed. The nuclei are large and the nucleoli prominent There are usually numerous mitoses.

It is important to emphasise that there are no nerve fibres present at this early stage of development within the developing epithelium of the otocyst. Underneath the basement membrane there are large, oval cells which may contain occasional nerve axons, suggesting that these cells are of the Schwann type

Development of the Embryonic Otocyst

Around the 6th day of embryonic development the surface epithelium tends to become better organised and occasional rudimentary kinocilia may make their appearance

There is now underneath the basement membrane, marked mitotic activity and large fascicles of non myelinated nerve fibres occupy the area (Fig 2) Some of the large cells seen under the light microscope consist of Schwann cells associated with multiple nerve axons (Figs. 3 and 4) These structures may be single or may form groups which correspond to the groups of large cells seen underneath the basement membrane under the light, or phase contrast microscope

From now on the innervation of the neuroepithelium proceeds at an obviously quickening tempo In the embryonic otocyst of 6 to 10 days single nerve fibres or fascicles penetrate the basement membrane and become established between the basal or supporting cells These cells seem to adopt the role of that of the Schwann cells, protecting and perhaps nourishing the multiple naked axons (Fig 7) The nerves anchor themselves at the base of the sensory cells, where they form single or multiple nerve endings



Fig. 1 F e-day-old embryo otocyst how ng the surface epithel um with indistinct cell membranes. There i no obvio nerv tissue nd no nerv endings within the surface epithelium in the early stages of dev lopment Compare with Fig. 10 14,000

(Fig. 10) Some of the nerv endings of the embryonic otocyst contain syn aptic vesicles and synaptic structures, including terminal bars make their appearance

The organelles of the cells have matured, nd the Golgi apparatus is well



F a 2 Six-d y-old mbry toc st with mit ti cell nd large numbers of nerv axons in the ubepithelial tissu 10,500

developed On the surface of the cells ciliary structures, stereocilia and kinocilia have reached full differentiation

Organ Cultures of the Otocyst

The process just described in the chick embryo can be seen repeated in tissue cultures of the isolated fowl embryo otocyst There appears to be a

Fig. 3. Seven-day-old embry tocyst showing fascicles of nonmy linated ners on ; also with nuclei of Sch an cell 7000.

ld embry otocyst Several Sch ann cells associated with l rge un-lated nerv xon 30,000.



Fi 2 Six-d y-old embryo otocyst with mitotic cell and larg numbers of nerv s ous in the ubepith li l tissu 10,500

developed On the surface of the cells ciliary structures, stereocilia and kinocilia have reached full differentiation

Organ Cultures of the Otocyst

The process just described in the chick embryo can be seen repeated in tissue cultures of the isolated fowl embryo otocyst There appears to be a



Fig. 7. Twelve-day-old tissue culture of an isolated fowl embryo otocyst. Fascicles of non-myelinated nerves lying between the cells of the basal layer of the neuroepithelium of the differentiated fowl embryo otocyst. Note nerves penetrating the basement membrane. × 14,000.

delay of a day or two in comparison. In the fully differentiated cultures of a total age of 10 days there are beneath the basement membrane capillaries, large fibroblasts and large cartilage cells. There are also numerous myelinated and non-myelinated nerve fibres beneath the sensory areas and

Fig 5 Twelve-day-old tissu culture of an isolated f wl embry otocyst howing Schwa cells with multiple nerve axon forming larg cell structures × 28,000

Fig 6 Tw l e-day-old tissu culture f isolated f wl mbryo otocyst Gr neuro



Fig. 7. T lve-day-old tissue culture f an isol ted f wl embryo otocyst. F scicles of non-myelinated nerves lying between the cells of the basal layer of the neuroepithelium of the differentiated fowl mbry otocyst. Note nerv penetrating the basement membrane 14,000

d lay f day or two in comparison. In the fully differentiated cultures of a total ge of 16 days there are beneath the basement membrane capillaries, larg fibrobl ts and large cartilage cells. There are also numerous myelinated and non myelinated nerve fibres beneath the sensory areas and



Fig. 8. Twelve-day-old tissue culture of the isolated fowl embryo otocyst. Multiple nerve endings at the base of a sensory hair cell. Sparse s n pt cal les 28,000.

multiple non myelinated nerve axons enveloped by one or more Schwann cells can be seen (Fig 5) The nerve fibres penetrate the basement membrane singly or in fascicles, and losing their myelin sheaths, progress between the basal cells (Fig 7) They ascend between the various cells of the neuroepithelium to reach the sensory cells where single budshaped nerve-endings or clusters of nerve endings are formed (Fig 8) Cup-shaped



Fig. 9. Twelve-day-old tissue culture of isolated fowl embryo otocyst. Synaptic region of hair cell which also contains terminal bar surrounded by vesicles. ×52,500.

nerve endings or calyces may also be formed in the vestibular region (Friedmann & Bird, 1967). There are very few synaptic vesicles, although terminal bars surrounded by vesicles are frequent (Fig. 9). The calyces may form invaginations filled with vesicles similar to synaptic vesicles. The surface of the sensory cell is by now fully equipped with stereocilia and kinocilia.



FIG. 10 T n-da -old embryo tocyst—n t ha cell with multiple nerv nding at the b se W ll de l ped cell m mbra es, t reocilia a d rgan ll (compare with Fig 1) × 8000

Neurones

The neurones forming the stato-acoustic ganglion have characteristic electron microscopic features (Fig 6) which differ from the ultrastructure of the large Schwann cells and similar structures observed underneath the

basement membrane and occur at some distance from the sensory epithelium

Filamentous or granular neurones can be distinguished (Fig 6) Some of the neurones seen formed a distinct axon hillock (Palay *et al* 1968) from which the nerve fibres originate.

DISCUSSION

Fell (1929–1930) was first o succeed in the cultivation of the isolated fowl embryo otocyst and demonstrated differentiation of the hair cells and some of the other epithelial elements to Corti s organ *in vitro* Fell was anxious to exclude any tissues outside the otic vesicle and her explants are a miracle of careful dissection There is no mention of the development of any nerve tissue in her observations indicating that the absence of the primordial stato-acoustic ganglion in the original explanted otic vesicle prevented the development of nerve tissue in the neuroepithelium.

We have achieved complete differentiation *in vitro* of the sensory areas of the fowl embryo ear using a modified watchglass method. We have soon recognised that "the inclusion of certain neurogenic elements plays an essential role in the differentiation of the sensory areas (Friedmann 1968) These are the stato-acoustic ganglion and eighth nerve primordia lying antero-ventrally near the neural crest and at some distance from the otic vesicle.

Proctor & Proctor (1967) ha e suggested that in the chick embryo the neurones of the acoustic ganglion developed from the neuroepithelial cells of the otocyst t about 71 hours and that following the establishment of the innervation of the sensory hair cells, at about 92 hours in the chick embryo, the auditory gangli n would migrate away from the otocyst.

This concept i based on light microscopic observations of the immature chick embryo otocyst We have re-examined the ultrastructure of the embryonic tocyst and found no support for this theory The large cells referred to by Proctor & Proctor are mostly Schwann cells. Moreover in the embryonic otocyst of 72 to 90 hours of embryonic age, there are no nerve xons within the developing epithelium.

The epithelial cells of the immature otocyst have no specific appearance nd there are no nerve endings or synaptic structures present these appear during the later tages of development of the neuroepithelium. There are no nerve fibres in the basal layers of the neuroepithelium either so prominent in the more advanced tages of development, when the number of the large Schwann cell ha increased to form groups underneath the neuroepithelium There may be found, in the differentiated otocyst occasional neurones among them. These however originate from the stato-acoustic ganglion and link up centrifugally with the neuroepithelium by means of outgrowing nerv xons. Terminal bars or deep invaginations surrounded by or filled w th ynaptic vesicles occur both in the 10-day old embryonic

otocyst and in the 12-day old cultures These synaptic structures are comparable with the *terminal bars* described by Smith & Sjöstrand (1961) in the hair cells of the cochlea of the guinea pig who have suggested that they could play an important role in the function of the auditory nerve. None of these structures occur before the later stages of maturation and differentiation of the neuroepithelial cells and tissues.

Our observations lead logically to the conclusion that the innervation of the otocyst and ceteris paribus, of the inner ear develop centrifugally from the neurones of the stato-acoustic ganglia which are products of the neural crest The neurones form axons which spread towards the neuroepithelium to reach the senory cells, associated with Schwann cells, which, according to Weston (1963) also originate from neural crest material

The outgrowth theory of course raises certain questions and one cannot but agree with Hughes (1968) lamenting that the outgrowth theory leaves *unsolved the embryological* problem of how it is that nerves connect with their right muscles and sense organs Thus, we are left with the vexed problem of how nerve fibres of the eighth nerve find their way to their appropriate destination in the labyrinth of the inner ear

CONCLUSION

The development of the innervation of the neuroepithelium of the developing fowl embryo otocyst has been re-examined under the electron microscope based on a longstanding study of the embryonic otocyst and of organ cultures of the isolated fowl embryo otocyst *in vitro* which provided an excellent system for these investigations

It has been shown that there are large Schwann cells, often enveloping large numbers of nerve axons in the subepithelial tissue which have to be distinguished from the neurones. It is pointed out that contrary to the suggestion of other authors there are no neurones in this area in the *early stages of development of the otic vesicle* Moreover nerve axons, nerve endings and other synaptic structures are also absent within the neuroepithelium *in embryos not older than 3 to 5 days.*

As maturation and differentiation progresses, large fascicles of nerve axons reach the *subepithelium from the more distant stato-acoustic* ganglion and link up with Schwann cells They proceed through the basement membrane and surrounded by various cells *of the neuroepithelium*, spread towards the sensory hair cells to form nerve endings and synaptic structures. The differentiation of the ciliary apparatus *of the hair cells appears* to be completed at about the same time 10 or 12 days in the embryo *in vivo* 12 to 14 days *in vitro*

The evidence supports the outgrowth therov and disproves the theory that the cells of the acoustic ganglion arise *through a division of the hair* cells themselves

ACKNOWLEDGMENTS

My sincere thanks are due to my collaborator and chief technician E. S. Bird, F.I.M.L.T. to Mr David Eastham and Mr J. D. Connolly for the excellent illustrations and Mrs A. L. Thornton for secretarial help. The research has been aided by grants from the Central Research Fund of the University of London and the National Deaf Children's Society as well as the British Empire Cancer Campaign for Research.

RÉSUMÉ

Le dévélopment de l'i nervation du neuro-épithélium sensoriel de l oreille interne et l'organe des termi tions des erf et des eurones en particulier a été ét dié au moyen de cultures d organes de l'otocyste embryonnaire de la poule et de l'embry n développant du poulet. On suggère qu elles prennent l'origi e dans les centres embry n iques dans le mésenchyme qui entoure l'otocyste et atteig ent les cellules neuro-épithéliales par de trous dans la capsule optique et la membrane basilaire. Ce fait est en co trad ction a ec la théorie que les neurones du ganglion développent des cell les neuro-épithéliales et se dispersent dans les tissus environants.

ZUSAMMENFASSUNG

Die Entwi klung des Innervation des Labyri ths wurde am Orga kulturen des Otocysts u d im Embry stud rt, mit beso derer Rücksi ht auf den Ursprung der Nervendigungen und der Ganglienzellen. Es wird die Theorie vertrete dass sie von den Anlagen im peri tischen Gewebe ihren Ursprung nehmen. Das widersprl ht der Theorie das die Ganglie llen des I nenohres on den neuroepithelialen Zellen entspra gen und in das mgebend Gewebe auswanderten.

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DISCUSSION

J Wersäll The excellent studies by Mr Friedmann clearly demonstrated the importance of "model organs" which can be used for different studies in inner ear research. The otocyst has turned out to be an excellent organ for studies of early developmental morphology and I hope that somebody will soon use it also for physiological tudies.

H K Kristensen As a supplement to the findings of Mr Friedmann I would like to mention our findings in a case of thalidomide intoxication

A girl aged 4 with no hearing had on tomography of the temporal bones no labyrinths. At autopsy no VIII nerves were found. On histological examination of the temporal bones there was on one s de an empty cavity in the place of the labyrinth On the other side there was also a cavity containing a well developed ampullary crest but no trace of nerve fibres below Th s could not be interpreted until today with the results of Mr Friedmann s experiments.

J Friedmann (Reply) I thank Mr Wersäll and Mr Kristensen for their encouraging remarks. Mr Kristensen s case seem to be an example of *in vivo* development of the inner ear in the absence of a central nervous link and control.

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INNERVATION PATTERNS IN THE ORGAN OF CORTI OF THE CAT

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The afferent and efferent nerve supply of the organ of Corti was studied after transection of the olivo cochlear fibres in the vestibular nerve the vestibular root, or at the floor of the fourth ventricle. After elimination of the efferent fibres by degeneration, the distribution pattern of the afferent fibres were evaluated with electron and light-microscopy. In the cat, all tunnel crossing radial fibres appeared to belong to the efferent innervation. The afferent fibres to the outer hair cells crossed the tunnel at the bottom as basilar fibres. The majority of afferent neurons ended at the inner hair cells and only a minority were associated with the outer hair cells. After selective transection of the crossed olivo cochlear fibres, a great number of the efferent endings at the outer hair cells degenerated but also the number of internal spiral fibres seemed to be reduced.

During the last few years I have published some unexpected results on the innervation pattern of the cochlear receptor of the cat (Spoendlin, 1967 and 1968). These results have not been generally accepted, especially by electrophysiologists, who have expressed some doubt about the reliability of these anatomical observations (Eldredge, 1967). Because of the fundamental importance of such questions, a reinvestigation was essential to provide additional, incontestable evidence for those certainly unexpected, innervation features where for instance the majority of the cochlear neurons appear to be associated with the inner hair cells and only a minority with the outer hair cells.

A careful examination of the terminal nerve fibres in the organ of Corti, with evaluation of their relative numbers, is only possible at an ultrastructural level, since most of these unmyelinated fibres escape observation under the light microscope.

It is well known that the organ of Corti is not only provided with afferent nerve fibres, but also with a rather extensive *efferent* nerve supply through the olivo cochlear bundle (Rasmussen, 1942). At the level of the modiolus, the efferent fibres are easily recognized as the intraganglionic spiral bundle (Fig. 1 A) but within the organ of Corti both *afferent* and efferent fibres intermingle and can hardly be distinguished. However their endings at the outer hair cells show clearly distinct features in electronmicroscopic preparations, the efferent endings being usually larger, filled with vesicles, and associated with a subsynaptic cysterna (Fig. 2).

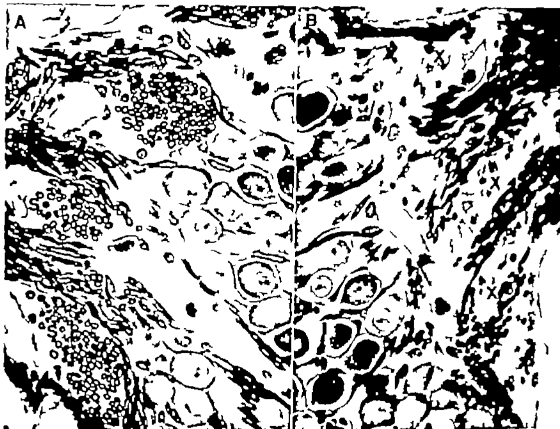


FIG. 1 Area of the intraganglionic spiral bundles of a cat in which the efferent cochlear fibres have been transsected on the right side 3 weeks previously by transsection of the vestibular nerve (*A*) Normal side with great bundle of numerous intraganglionic spiral fibres (*S*) (*B*) operated side The intraganglionic spiral fibres are practically entirely missing (X) and only Schwann cells can be seen in their location There is no appreciable reduction of spiral ganglion cells

In order to study the distribution pattern of the afferent nerve fibres within the organ of Corti, we induced degeneration of the efferent fibres by transsection of the olivo cochlear bundle in the vestibular root (Spoendlin & Gacek, 1963) or in the vestibular nerve (Spoendlin 1967) Early signs of degeneration within the efferent endings in the form of concentrical membranes around groups of mitochondria, were found 12 hours after the lesion (Fig 3) After 2 weeks, degeneration was usually complete and, with the exception of occasional small cell debris, nerve endings and fibres had completely disappeared (Fig 4) Judging from degeneration studies in the chinchilla by Smith & Rasmussen (1963) there might be considerable species-differences in the time course and mode of degeneration

The fact that all richly vesiculated nerve endings at the outer hair cells, and most inner spiral fibres (Fig 12) belong to the efferent innervation is, I think, today accepted by most people since at least in the cat they all degenerate after transsection of the olivo cochlear fibres Subtotal degeneration of these endings was shown by Iurato (1962) in the rat and by Smith & Rasmussen in the chinchilla (1963) after lesions of the olivo cochlear



Fig. 2. Nerve endings at the base of an outer hair cell (*H*) of a normal animal. The efferent endings (N) are filled with synaptic vesicles and associated with a subsynaptic cistern on the hair cell membrane. The afferent terminals (*A*) contain only very few vesicles and appear to have single synaptic membranes. (Fixation with 1% OsO4 in phosphate buffer.)

fibres in the brain stem. However, as we reported earlier, the tunnel crossing radial fibres also disappear after such lesions manifesting their efferent nature. The question whether all tunnel radial fibres are efferent or not is in fact of crucial importance for the study of the distribution of the afferent fibres. Therefore we will try to present as much evidence as possible that in the cat practically all tunnel radial fibres really belong to the olivo cochlear efferent system.

In experiments designed to provide such evidence two main possible errors have to be taken into account. The lesion of the olivo cochlear fibres must be complete and should not cause damage to the afferent cochlear

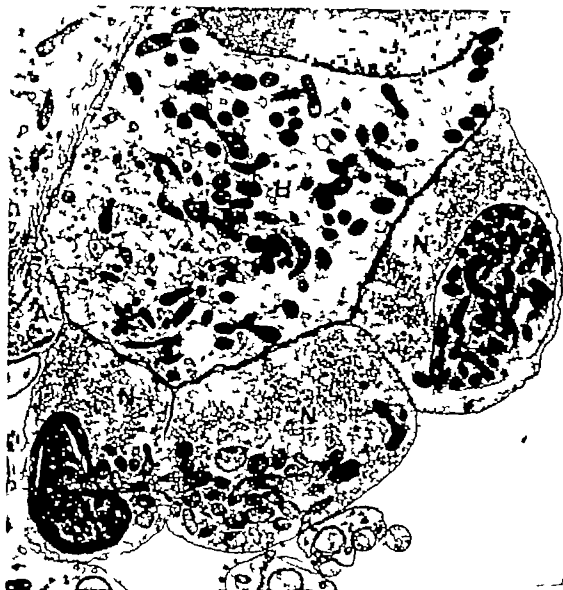


FIG 3. N rve nding at th base f an ute h ir cell (*H*) f a cat in which the contra l teral livo cochlea fibres ha e been tran sected 12 hours previ u l by a midline lesion at the floo f the 4th nt icl Alre d t thi time ig f deg eration in the efferent nerv ndings (N) re clearly see l form of conce tri m mbranes around groups of mitocl ondria It is ery marked l th l wer left and l n initial tat at th uppe right f the picture One aff rent t rminal i seen at (*A*) (Fixation with 2% OsO4 in collidin buffer)

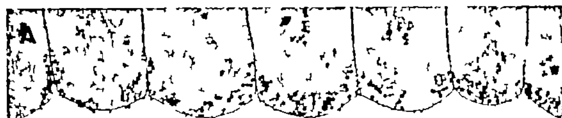
neurons If the lesion is made by transsection of the vestibular nerve in the internal acoustic meatus, all olivo cochlear fibres are usually included—however some afferent neurons can be damaged or the lesion may interfere with the cochlear blood supply Such errors can be controlled by an evalua tion of the number of spiral ganglion cells or the myelinated nerve fibres in the osseous spiral lamina If these numbers are not appreciably reduced, important damage of the cochlear afferent neurons can be excluded. The efficiency of the lesion can also be checked by degeneration of the intra

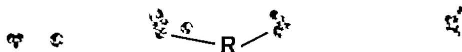


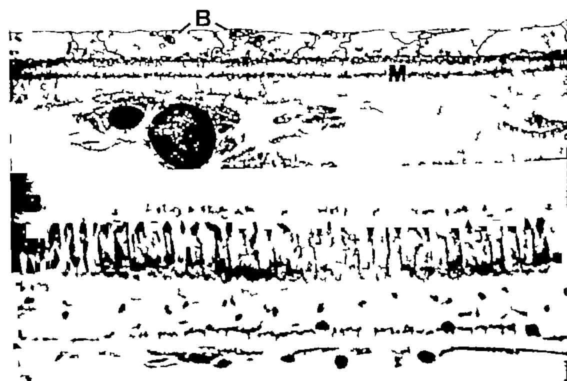
Fig. 4. Lower end of outer hair cell f cat i which the lh cochlear fibres ha been tra sected 3 day previou l The fferent ers endings (\) re entirely des-l tegrated d only some cytological debris re still present. The subsynaptic cysterna long the hai cell membrane ppears t be normal T fferent nerv terminals (*A*) how no cha ges.

ganglionic spiral fibres (Fig 1 *B*) An ther cause of error is the difficulty of evaluating the totality of all tunnel radial fibres.

Different approaches were used to control these pos ible errors. The ears of on anim l where the vestibular nerve was cut on one side 1 month previously were w ked up in the way of normal temporal bone histology using celloidin embedding and 15–20 micron thick sections. Every section was carefully xamined In the normal control-ear 2–5 radial tunnel fibres were clea ly seen in each organ f Corti of each section. In the operated ear they were mi sing and only one questionable remaining tunnel radial fibre was found throughout the entire cochlea. The intraganglionic spiral bundle was degen rated and the ganglion cell population in the spiral ganglion was


A


R


B
M



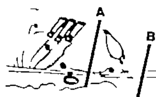
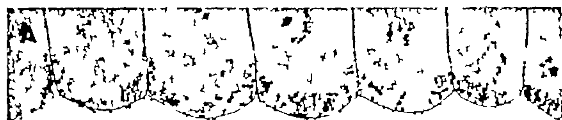
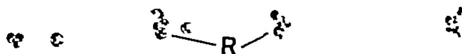
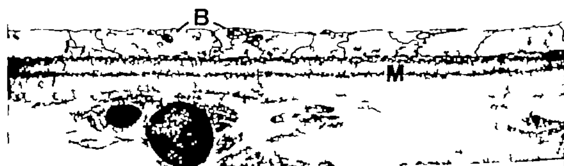


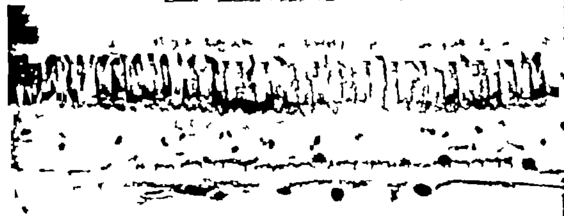
FIG. 6. (A) Light microscopic pict re f tangential secti t the t nnel of imal i which the oliv cochlea bundles ha been tran sected in the estibula ners 4 weeks previou ly Th tunnel space (T) ppears entirely empty nd t nel radi l fibres are ymore seen. (B) T genti l section through the beginning of th osseou piral lami f the same re as in section A (see schem tic drawing) which show that the number of the myelinated ners fibres is not ppreciably reduced.

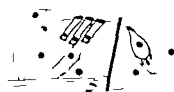
FIG. 5 (A) Electron-micrograph of tangential sectio through the tunnel of Corti as indicated in the schematic drawing on the left. The f scicles of the tunnel crossing radial fibres (R) re trans ersly sec ti ned nd learly seen how they lay entirely free i the t nnel On the other hand there re the basila fibres (B) at the bottom of the t nel, i most cases embedded in i aginations of the pilla cells. (B) Phase-contrast picture of larger tangential section through the t nel of Corti. Here, the f scicles of the tunnel radial fibres are still clearly isible as black pots.


A


R


B
M





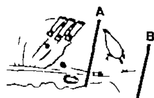


FIG. 6 (*A*) Light microscopic picture of tangential section t the tunnel f an imal i which the l cochlea bundles ha bee transected i the estibular ner 4 weeks previously. The t nnel pace (*T* ppears entirely empty nd t nel radi l fibres are y more seen. (*B*) Tangential sectio through the beginning of the osseous piral lamina of the sam rea as i section *A* (see schematic draw ng) which show that the mber of the my li-nated ner fibres is not ppreciably reduced.

FIG. 5. (*A*) Electron-micrograph f ta gential section through the t nnel of Corti indicated i the schematic drawing on the left. The fascicles f the t nnel crossi g radial fibres (*R*) re tra sversly sec tioned nd clearly seen how they lay entirely free i the t nnel. On the other hand there are the basila fibres (*B*) t the bottom of the t l, i most cases embedded i inv ginations of the pilla cells. (*B*) Phase-contra t picture f larger tangential section through the t nel f Corti. Here the f scicles of the t nnel radial fibres are still le rly visible black spots.

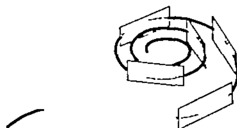


FIG. Sch m tic drawing showing how th tangential sections f the organ of Corti has been tak n in ord to evaluate as great portions of the organ of Corti possible in phace contrast microscopy In this w y about / of th ntire length of the organ of Corti of no cochl a can be examined.

normal showing an intact afferent nerve supply to the cochlea. In this way the entire cochlea could be examined, but the tunnel radial fibres are not very conspicuous in ordinary histological sections and an exact evalua tion is hardly possible With electron microscopy on the other hand, only very restricted areas of one cochlea can be examined

Tangential sections through the tunnel of Corti where the tunnel is sectioned in a longitudinal direction, were found to be the best way to cover greater portions of the organ of Corti in one section and to visualize all tunnel radial fibres within the limits of each section In these sections, the tunnel radial fibres appeared cross sectioned in fascicles of two to five fibres, presenting great variations in caliber We measured diameters from 0 1 up to 0 6 microns (Figs 5 A 8 A) In larger thicker sections for phase-contrast microscopy these small fascicles were still clearly visible as black spots and much larger areas of the organ of Corti could be examined (Fig 5 B) One month after transsection of the olivo cochlear bundle these tunnel fibres had gone (Fig 6 A) Taking some sections through the osseous spiral lamina of exactly the same area, the condition of the afferent neurons was controlled and no signs of degeneration or reduction of their number could be seen (Fig 6 B)

From one cochlea several tangential sections could be obtained from the first and second turn which allowed us to cover about 1/2 of the entire cochlea (Fig 7) The cochleas of three animals with previous section of the olivo cochlear fibres were systematically examined in this way and in four other animals we evaluated one or two tangential sections of the basal turn In all these examined specimens the tunnel radial fibres disappeared practically completely and there was no appreciable reduction of nerve fibres in the osseous spiral lamina, indicating an undamaged afferent nerve population

Thus we confirm our earlier observations, that in the cat all tunnel radial fibres belong to the efferent olivo cochlear system

The only remaining fibres crossing the tunnel are the basilar fibres which therefore have to be considered as afferent neurons (Figs 5 A 12) The number of these basilar fibres reaching the area of the outer hair cells

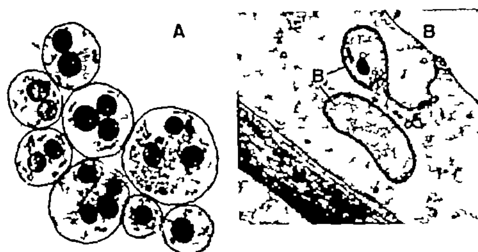


Fig. 8. (A) High magnification of a section of tunnel radial fibres showing their large variation in diameter. (B) two basilar fibres (B) entirely embedded in deep invaginations of the pillar cell above the basilar membrane (M).

is surprisingly small. They can be counted easily as they penetrate between the outer or inner pillars. At the bottom of the tunnel they are usually embedded in invaginations of the pillar cell (Fig. 8 *B*) and most of them take a short spiral course before they pass between the outer pillars. In tangential electron microscopic sections these small fibres could be seen cross-sectioned between the outer pillar feet (Fig. 9). In several sections of the first and second turn of the cochlea of different animals we evaluated the numbers of these fibres over a distance of approximately 1.5 mm, which correspond to about 200 outer pillars. We found a ratio of 180 nerve fibres to 200 outer pillars, which is an average of little less than one fibre per one pillar or a total of about 3000–5000 fibres. On the one hand, these relatively few basilar fibres appeared to be the only ones which were destined for the outer hair cells. On the other hand, practically all outer spiral fibres remained unchanged after elimination of the efferent nerve supply indicating that they belong to the afferent neuron (Fig. 10). They are the continuation of the basilar fibres, which turn basalwards after having passed the outer pillars and run in a spiral direction over a considerable distance before they give off their terminal collaterals to several outer hair cells. The relation between the number of outer spiral fibres and basilar fibres penetrating between the outer pillars allows an estimation of the average spiral extension of the outer spiral fibres which is approximately 0.6 mm (Spoendlin 1968).

Further evidence for such a predominant nerve supply to the inner hair cells was provided by reconstruction of the area between the habenula perforata and inner hair cell in animals where only the afferent dendrites are present, the efferents being eliminated by previous transsection and degenera-



FIG 9 Electron micrograph of t ng ital section through the outer pill feet s I dicated in the sch m tic drawing At thi l el the number f basi lar fibres (*F*) which pen trat between the outer pill rs to rea h th area of th outer hai cells can be counted These fibres re not very regularl distri buted. At s m places two o three fibres ru together at th rs only n fibr l vs between t pilla cell and again at ther places n fibre at all is f u d between two pillar cells. If such sections are val ated ver great distances w find an a r ag f a littl less than one afferent fibre between two pilla cell

tion of the olivo cochlear fibres. In a series of several hundred sections, each individual fibre was followed from the habenula to its ending at the inner hair cell or to its passage between the inner pillars For a better demonstration, we made a graphic reconstruction of the area of two inner hair cells on the basis of such serial sections Six representative sections of this series were chosen to reconstruct the area in a drawing The sectional plans used to build up this reconstruction are exact copies of the original elec tron micrographs from the series (Fig 11) The fibres enter the organ of Corti in well packed bundles of about 10–20 fibres per habenular opening These nerve fibre bundles are maintained for a short distance after which the fibres disperse between the supporting cells The majority continue



Fig. 10. Outer spiral fibres (S) between the three rows of Deiters cells (D) in [illegible] where the [illegible] cochlear efferent fibres had been transected [illegible] weeks previously. There is no appreciable reduction of the number of outer spiral fibres [illegible] that they are [illegible] nerve fibres.

in an essentially radial direction to reach the closest inner hair cell. Only very few fibres take a short spiral course *before they penetrate between* the inner pillars to continue as basilar fibres or before they end at one of the neighbouring inner hair cells. In reconstructions covering the area of *five inner hair cells we found out of 20 afferent fibres* entering the organ of Corti only one or two turning outwards to the outer hair cells. These numbers are consistent with the number of basilar fibres which pass between the outer pillars. The great majority of all fibres end unbranched with a single nerve ending at the inner hair cells. Each ending presents a typical synaptic complex, characterized by *a marked thickening of* the postsynaptic membrane, a minor accentuation of the presynaptic membrane, and a pronounced synaptic bar surrounded by vesicles within the *sensory cell's cytoplasmic (cf. Smith & Sjöstrand, 1961)*.

All these observations give, I think, incontestable evidence that the great majority of sensory neurons are associated with the inner hair cells and only a minority with the outer hair cells, even though this might be against prevailing opinion.

If we count 50 000 cochlear neurons in the cat (Gacek & Rasmussen, 1961) only about 10% of them would be associated with the outer hair cells, where each fibre is connected with a considerable number of sensory cells. *Kellerhals & Engström (1968) found among the spiral ganglion* cells in guinea pigs approximately 10% ganglion cells which were different from the majority. Whether these correspond with neurons connected to the outer hair cells remains to be clarified.

The important functional significance of such a fibre distribution is evident. Considerations as to its possible functional implications are published elsewhere (Spoendlin, 1967 and 1968). It could for instance provide a greater dynamic range for the organ of Corti, in such a way that the nerve *fibres with multiple endings* at the outer hair cells would be the most sensitive ones, whereas the fibres with single endings at the inner hair cells would have a higher threshold. Some sort of mechanism to increase the dynamic range *of the cochlear receptor-organ has to be postulated because* in single neurons the range of intensity between the first increase in rate of discharge up to the maximal rate of discharge is only about 30 dB, whereas the range between thresholds of the most sensitive fibre unit *and the least* sensitive unit at a given characteristic frequency may be 50 or 60 dB.

Fig. 11. Graphi rec nstructi f the re of two t rnal hai cell f n an m l l wh ch th h ochl eff rent fibres ha been tra ected i th estibula ers 4 week pre l u l. The reconstructi mad the ba i f a seri of several hundred sect s 5 represent ti les ls re sh wn ref re t poi t f th drawing. The secti n l pla n th drawi g re ct copies f th co e po di g electron micrograph out f th ri. On electro m crograph i hown n sampl. It correspo d to the section l plan di wing 5. *It i l ly seen how th great m jorit f* afferent dendrites i directed towards the int rn l hair ll nd nds there with a si gl terminal.

1

2

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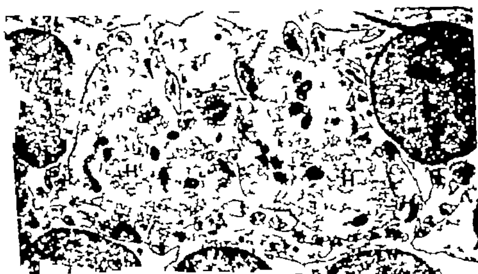




Fig 12. Surview of th rea below th int rnal hair cell (*H*) A bundle f nerv f bres merges from the habenula ope ing (*O*) The maj ity of these fibres are affere t den drites (*D*) Below the internal hair cell there re great numbers of very small inner piral fibres (*S*) Adjacent to th inn pillar a simila group of nerve fibres forms the tunnel piral bundl (*T*) At the lowe l ft two ba la fibres (*B*) re seen s they start thei course cross the floo of th tunn l They appea to be the fferent dendrites for the outer h ir c lls.

(Davis, 1968) There seems however to be a gradual transition from the more sensitive neurons associated with the outer hair cells to the less sensi tive neurons associated with the inner hair cells since Nelson Kiang (1965) in his studies on single primary auditory units, finds no outstanding group of especially sensitive fibres

This innervation pattern, as it revealed itself in our anatomical studies, is not always in good agreement with electrophysiological observations Thus Fex (1968) mentions two studies on single nerve fibres of the cochlear nerve of the cat where the activity of nearly all fibres could be inhibited

by stimulation of the crossed efferent fibres, which according to Iurato (1962) innervate only the outer hair cells in the rat Here species-differences and the difficulty of evaluating the number of internal spiral fibres might play a role In five cats where the crossed olivo cochlear fibres have been cut by means of a midline lesion, we found the great majority of efferent fibres in the intraganglionic spiral bundle degenerated and we got the impression of a clear reduction of the internal spiral fibers. However an exact evaluation of these experiments will be published later

RÉSUMÉ

L'i nervation efferente et afferente d l'orga de Corti est étudié dans le microscope électronique après lesion des nerfs suivants des fibres olivo-cochléaires dans le nerf ou dans la raci e vestibulaire du nerf cochléair Les résultats principales portent sur la différen iati n de la distribution des fibres olivo-cochléaires croisées et non croisées et sur la distribution des eurons cochléaires dans l'organe de Corti, dont la majorité parait d'être destinée pour les cellules ciliées internes.

ZUSAMMENFASSUNG

Die afferent u d die efferente Innervation des Cortischen Organes wird untersucht nach Durchtren ung der oli o-cochle:ren F sern im Nervus estibularis, in der Vestibulari wurzel oder am Boden des vierten Ventrikels. N h Elimination der efferenten Fasern durch Dege eration kann das Verteilu gsmuster der afferenten F sern im Cortischen Org mit Hilfe von Elektronen und Licht mikroskopie untersucht werden. Bei der Katz scheinen alle tun elkreuzenden Radialfasern zur fferenten I nerv tion zu gehören. Di fferenten Fasern kreuzen den Tunnel m Boden ls Basilarfasern. Di grosse Mehrheit der afferenten Neuronen endigt an den i eren Haarzellen nd nur ein Mi derheit zieht zu den äusseren Haarzellen Bei selekti er Durchtrennu g der gekreuzten olivo-cochleären F sern degeneriert i e Grosszahl der efferenten Endign gen an den äusseren Haarzellen ber uch d inneren Spiralf sern schei en dezimiert zu werden.

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Fi 12. Surview of th are bel w the internal hai cell (*H*) A bundle f nerve fibres merges from th habenula openi g (*O*) Th m jority of these fibres are afferent dendrites (*D*) Bel w the i t rnal hair cell there are great numbers of v ry mall inner piral fibres (*S*) Adjacent t th inner pill a imilar group of nerve fibres f rms the tunn l piral bundl (*T*) At the l wer left two ba ila fibres (*B*) are se as they tart th l co rse across th floor of th tunnel They ppea to be th ff rent dendrites f the uter hair cells

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COMPORTEMENT DE QUELQUES PHÉNOMÈNES AUDITIFS EN HYPOXIE

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L.A. f it des recherches sur le comportement de certains phénomènes auditif en hypoxie (respiration pend nt 5–10 min de mélanges au 7,6% et u 8,6% d'O_2 en N) Les tests ont été les suivants sensatio spatiale déterminée par deu tons d égale intensité mais a ec une petite diffé rence d fréquence le test d discrimination dychotique les tests vocaux avec distorsi n ol accélérée et interromp e L'hypoxie a déterminé des altérations particulièrement ignificatives pour les sensations spatiales et de mineur degré pour la voix distordue et accélérée. L.A. est de l'avis qu un diminution d tension d'O_2 détermine de altération f c tionnelles surtout pou les procés qui se servent de circuits polysynaptiques et d ne intégration neurale précise

On sait par une série de recherches que le flux circulatoire cérébral représente une entité relativement constante et qu'il ne ressent presque pas les variations de tension partielle de l'O_2. Reivich, par example, a démontré en 1964 que des changements contrôlés de la vitesse de flux hématique cérébral de 20 à 100 ml/min pour 100 gr de cerveau, produits par des variations contrôlées de la pression partielle de l'CO_2, ne sont pas influencés par des changement de la tension partielle d l O hématique entre 60 et 380 mmHg. Un grand changement de pO parait ainsi compatible avec un flux hématique cérébral constant. Pour l'h mme aussi la respiration d'O_2 pure cause seulement une petite diminution (13%) du flux hématique cérébral (Kety 1953)

On sait par contre qu'il y a un gradient très rapide de la tension partielle de l'O ntre les vaisseaux et les cellules cérébrales 33 mmHg dans les vaisseaux piaux et 1 mmHg au niveau intracellulaire pour Chance et autres Aa. Des gradients similai es ont été démontrés par Misrahy entre les vaisseaux de l strie asculaire et les cellules de l rgan de Corti.

Il faut enfin rappeler qu la courbe d'utilisation de l O_2 par les cellules augmente avec l ugmentation de l acti ité fonctionnelle d'une façon non l né ire Da ies & Crenell, 1962 Chance t al 1962) par un système enzymatiqu cellulaire très efficient qui permet une respiration cellulaire normale même ec une relativement basse tension d'O_2.

Rech rches nalogues sur l oreill interne ont été faites par T unoo & P rlman (1965)

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E N T D partment the University of Züri h Zürich Swit erland

DISCUSSION

H Engström If as there is reason to believe, Mr Spoendlin s observations are correct, and if there is no factor involved that confuses the pattern of degeneration I do believe that his findings are of extreme importance for the understanding of the cochlear function We have mainly used guinea pigs and monkeys in our studies and it is clear that these animals differ from the cat, but I cannot say yet if the same principle of extremely rich innervation of the inner hair cells is present or not. Certain information from humans indicates that if inner hair cells are damaged this has greater importance for the degeneration of the nerve fibres in the spiral osseous lamina than a comparable degeneration of the outer hair cells. This would be in good agreement with Spoendlin s findings.

A Meyer um Gottesberge Die eindrucksvollen Bilder von Hr Spoendlin zeigen die dichte Innervation des inneren Haarzellen Könnte es sein, wie ich 1948 vorschlug, dass das Recruitment davon abhängt, dass die äusseren Haarzellen abgenerieren die inneren jedoch erhalten sind und infolge ihrer höheren Schwelle und ihrer dichten Innervation den Lautheitsanstieg beziehen?

H Spoendlin (Reply) to Mr *Engström* I am fully aware of the fact that there are considerable differences between animal species. In guinea pigs, for in stance there are many afferent fibres crossing the tunnel at a higher level. The separation of afferent and efferent fibres is especially clear in the cat, which was one of the reasons why I chose this animal. I tried to get as much evidence as possible for these innervation-patterns and I hope that now others are repeat ing similar experiments to reinforce the evidence

To Mr *Meyer um Gottesberge* Our findings would in fact support the view that recruitment occurs when the outer hair cells are missing The extensive ramifications of the afferent fibres at the outer hair cells, where one fibre innervates a great number of outer hair cells, and the observation that each inner hair cell is innervated by a great number of unramified afferent neurons, suggests a higher threshold for the activation of the nerve fibres at the inner hair cells. Once this threshold is reached however a great number of fibres are probably activated together

TABLEAU 3. Résultats obtenus pendant la respiration avec un mélange pauvre en oxygène (8,6% d'O_2)

Fréquence	Type	Après 2 minutes	Après 5 minutes
125	1 3	5–0	3–1
250	2	5–0	3–4
500	3–4	0	4–5–0

conditions optimales on a la sensation d un déplacement de la source sonore tout autour de la tête Autres fois le déplacement subjectif est limité au devant, au derrière ou au dessus de la tête nous pouvons enfin avoir une sensation de déplacement limitée à des îles ou des îlots alternativement à droite et à gauche de la tête ou finalement on peut avoir simplement une vague sensation de déplacement sans en préciser la localisation (Tableau 2)

Les tests susmentionnés ont été faits sur des sujets de moins de 35 ans, sains, avec fonction auditive normale Après les contrôles préliminaires les sujets respiraient des mélanges pau res en O_2 (respectivement 8,6% et 7,6% d'O_2 en N) Les déterminations étaient faites préablement, après 2 et après 5 minutes de respiration du mélange Les résultats sont indiquées sur les Tableaux 3 et 4 et sont exprimés par les chiffres qui caractérisent les différents types de sensation sur le Tableau 2.

Si on analyse ces résultats on voit après 2 min la disparition des sensa tions spatiales plus fines (comme ce déplacement du son autour de la tête ou bien au devant, au derrière ou au dessus de la tête) Les sujets étudiés n ont pas de sensation de mouvement du son dans l espace ou bien ils ont seulement une sensati n de déplacement de la source sonore incomplète et vague Après 5 min de respiration des mélanges pauvres en O on a une certaine reprise des sensations spatiales, tout de même sans rejoindre les aleurs normales. La reprise de sensation est plus évidente avec la re spiration du mélange au 8,6% d'O_2 plutôt qu avec le mélange au 7,6% d O_2.

Une deuxième série d observations a été faite avec l'emploi du test de discrimination dychotique qui est constitué par une double série de mots et d hiffres entr ux différents, qui sont envoyés séparément et au même

TABLEAU 4 Résultats obtenus pendant la respiration avec un mélange pauvre en oxygène (7,6% d'O)

Fréquence	Type	Après 2 minutes	Après 5 minutes
125	1 3	5–0	4–5
250	2	–0	4–5
500	3–4	0	5–0

TABLEAU 1 *Caractéristiques des ondes employées*

I dB	F	F	Δf/f %	Mo
40	125	125 4	0,32	1/5
40	250	250 4	0 16	1/5
40	500	500 4	0,08	1/5″
40	1000	1000 4	0,04	1/5

Cependant il y a des conditions ou la réduction de tension de O_2 au dessous d un certain niveau critique provoque une diminution de la respiration cellulaire Les altérations fonctionnelles qui en suivent sont particulièrement évidentes pour ces phénomènes qui se réalisent par des circuits polineuronaux et qui demandent une intégration des activités neurales.

A la Clinique ORL de l Université de Turin nous sommes en train de faire une série de recherches par différentes méthodiques sur le comportement de quelques phénomènes qui intéressent la voie acoustique centrale Nous avons étudié les altérations induites par l Hypoxie sur certains tests et nous avons recherché s il y a des rapports avec les résultats observés chez des sujets avec des altérations du système nerveux central

Un premier groupe de recherches a été fait sur l ainsi dite sensation spatiale acoustique déterminée par deux sons qui agissent sur les deux oreilles avec égale intensité (40 dB au dessus du seuil) et une très petite différence de fréquence (moins du 0.5 %) On obtient ainsi une période de modulation constante et inférieure à la minute seconde Les fréquences employées ont été de 125 250 500 et 1000 Hz (Tableau 1) Des recherches préliminaires faites par Menzio et Schindler sur des jeunes auditivement normaux ont démontré qu on peut réaliser des différentes sensations spatiales plus ou moins nettes selon les sujets et les bandes de fréquence employées. En

TABLEAU 2 *Types de sensation*

1		Sensation spatiale complèt u tour de la tèt
2		Sensation spatiale complète Au devant Au derrière Au dessus de la tête
3		Sensation spatiale incomplète (grosses îles droite t g uche)
4		Sensation spatiale incomplète (îlots droite et gauche)
5		Sensation spatiale incomplète Pas d localisation

TABLEAU 3 *Résultats obtenus pendant la respiration avec un mélange pauvre en oxygène (8,6% d'O)*

Frequence	Type	Après 2 minutes	Après 5 minutes
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4	Sensati n spatiale incomplèt (llot droite et ga che)
5	Sensation spatiale incomplèt Pas d localisation

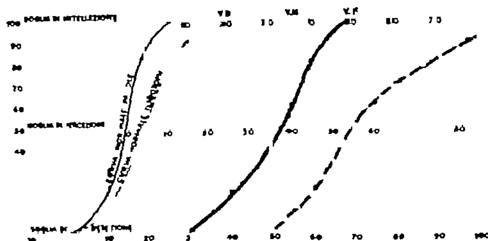


Fig. 2. Hypoxie — voix accélérée ——— Normal ; courbe en hypoxie

sitent des procès d intégration neurale extrêmement délicats et précis. C'est la condition typique des sensations spatiales déterminée par deux sons avec petites différences de fréquence pour lesquelles en effet le jugement se base sur les seules différences de phase Il faut rappeller à ce propos que par ce test on peut déceler des altérations précoces et ainsi de valeur dia gnostique chez les syndromes de démiélinisation, les presbyacousies et les traumatismes craniaux même légers. Des altérations, peut être moins évi dentes mais toujours significatives, ont été vues avec les tests de la voix avec distorsion et de la voix accélérée, qui se modifient surtout pour les lésions de l'aire acoustique et pour les lésions temporales profondes.

Des modifications inconstantes et modestes ont été rélevées par le test dychotique pas d altérations il y a eu par le test de la voix interrompue, qui indique généralement des lésions au ni eau mesencéphalique. En con clusion il nous semble justifié croire que l'hypoxie peut déterminer des altérations fonctionnelles pour les procès qui se passent sur des circuits poli synaptiques surtout au niveau cortical ou soucortical

SUMMARY

In respiratio for 5–10 min in mixture of 7.6% and 8.6% oxygen in N, the author has made the f llowing tests: Acoustical space sensation by sounds of equal int nsity and frequency difference dychotic test nd vocal tests by distorted, accelerated, and i terrupted voice Significant alteration were noticed for space sensation and less for accelerated and interrupted oice Oxygen dimi nution should determin functional modification for auditory processes which involve polysynaptic pathw ys and precise neural integration

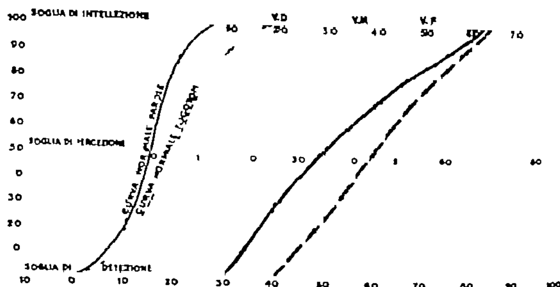


Fig. 1 Hypoxie – voix philtrée ——— Normale; - - - courbe en hypoxi

temps aux deux oreilles à une certaine intensité Par l examen des réponses on peut établir un pourcentage d intelligibilité du test qui devrait donner des indications sur les capacités de discrimination et d intégration acoustique à un niveau supérieur La respiration des mélanges pauvres en O_2 ne détermine que peu de modification aux réponses du test la comprehension des messages verbaux est en effet toujours supérieure au 85% des mots transmis Pour un seul cas, avec le mélange au 7 6% d O_2 le pourcentage est descendu au dessous du 50%

Une troisième série d observations a été faite par les tests de la voix avec distorision voix accélérée et interrompue Les sujets de cette série respiraient les mélanges pour 10 min

Et voici les résultats

Voix avec distorsion Modification d intelligibilité à basse intensité En effet le seuil de detection et de perception étaient rejoints à une intensité de 15–20 dB supérieure aux épreuves effectuées avec respiration d air normal tandisque le seuil d intellection était rejoint à la même intensité qu en conditions normales (Fig 1)

Voix accélérée Modifications de l intelligibilité à toutes les intensité On a jamais pu rejoindre le 100% d intelligibilité avec la plus grande intensité de l audiomètre les courbes ont toujours une forme à plateau (Fig 2)

Voix interrompue Pas de modification

Les observations que je viens de décrire ont une signification plutôt complèxe et difficilement on peut en tirer des considérations de caractère conclusif Cependant on peut affirmer que les altérations plus importantes déterminées par une chute de tension d O_2 se réalisent pour les tests qui néces-

(ARTICULATION CURVE)

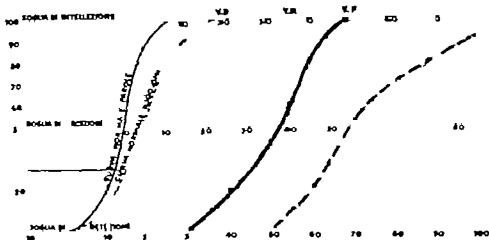


Fig. 2. Hypoxie — el accélérée ——— N rmale; — — courbe en hypoxie

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SUMMARY

In respiration for 3-10 min in a mixture of 7.6% and 8.6% oxygen in N, the uthor has made th f llowing tests Acoustical space sensation by sounds of equal intensity and frequency difference d) hotic test nd vocal tests by distorted ccelerated a d interrupted voice Significant alterations were noticed for space se sation d less for ccelerated nd interrupted voice Oxygen dimi-nution should determin functional modifications for auditory processes which involve polysynaptic pathw ys and precise neural integration.

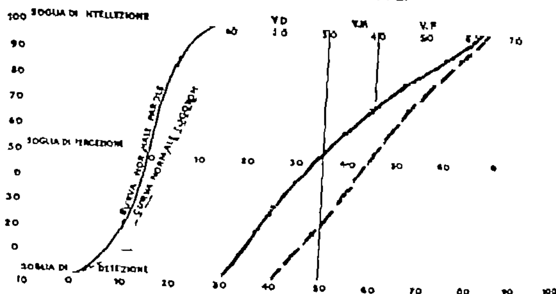


Fig 1 Hypoxie – voix philtrée ——— Normale - - - courbe en hypoxie

temps aux deux oreilles à une certaine intensité Par l examen des résponses on peut établir un pourcentage d intelligibilité du test qui devrait donner des indications sur les capacités de discrimination et d'intégration acoustique à un niveau supérieur La respiration des mélanges pauvres en O_2 ne détermine que peu de modification aux réponses du test la comprehension des messages verbaux est en effet toujours supérieure au 85% des mots transmis. Pour un seul cas, avec le mélange au 7 6% d O_2 le pourcentage est descendu au dessous du 50%

Une troisième série d observations a été faite par les tests de la voix avec distorision, voix accélérée et interrompue Les sujets de cette série respiraient les mélanges pour 10 min

Et voici les résultats

Voix avec distorsion Modification d intelligibilité à basse intensité En effet le seuil de detection et de perception étaient rejoints à une intensité de 15–20 dB supérieure aux épreuves effectuées avec respiration d air normal tandisque le seuil d intellection était rejoint à la même intensité qu en conditions normales (Fig 1)

Voix accélérée Modifications de l intelligibilité à toutes les intensité On a jamais pu rejoindre le 100% d intelligibilité avec la plus grande intensité de l audiomètre les courbes ont toujours une forme à plateau (Fig 2)

Voix interrompue Pas de modification

Les observations que je viens de décrire ont une signification plutôt complèxe et difficilement on peut en tirer des considérations de caractère conclusif Cependant on peut affirmer que les altérations plus importantes déterminées par une chute de tension d O_2 se réalisent pour les tests qui néces

the most highly vasculari ed parts of the whole central nervous sy tem. In my own i vestigation of children dying from asphyxia I found a severe cell loss in these nuclei, up to more than 60% of the cells were lost in the dorsal nucleus. Changes of this order were not found anywhere else in the auditory system.

P Men lo (Réponse) à Mr *Holmgren* Il est probable que les altérations, dan la compréhension d'un message verbal, caractéristiques des patients presbiacou siques soumis à hypoxie solent dues u fait que les modifications anat mo-pathologiques et fonctionnelles du vieillard sont localisées soit à niveau de l'organ de Corti, soit à niveau des voies auditives centrales. Dan ces conditions l'hypoxie peut déterminer des altérations remarquables dans les processus d'intégration neuronale

Un appui à cette interprétation est répresenté par le fait que l'hypoxie ne deter mine pas des modifications du seuil pour les tons purs, mais dans la compréhen sion des mots, des logatomes et des phrases.

En ce qui concerne les erreurs dans la dactylographie, déterminés par l'hy poxie, je crois que la chaine des phénomènes (idéatif et d'élaboration motrice) qui sont i dispensables pour qui pratique la dactylographie et qui nécessitent l'action de circuits pol euronaux très compl es, solent facilement troublés par une modification de la tension partielle d'O, et par la conséquente altération métabolique

Et enfin, pour ce que concerne la troisième questi n, je pense que les modifications observées à l'audiometrie vocale soient secondaires à variations fonctionelles soi à niveau cochleaire que soucortical.

à Mr *Hall* Je suis d ac ord avec lui sur l'importance des noyaux cochléaires, surtout du noyau accessoire qui a fait l'objet d'études particullèrement impo tants, surtout au point de vue électrophysiologique et anatomopathologique.

J pense toutefois que les resultat obtenus pa les différentes techniques, et par celle que j'ai référé en précédence, f t supposer que l'hypoxie détermine des modifications f nctionnelles à des niveaux différents, mais surtout à des niveaux corticeaux et sous-corticeaux. Seulement des recherches systématiques, électrophysi logiques, linlques, expérimentelles et farmacologiques (substances neurotropes) pourront nous do ner des renseignements précis sur l'importance des différentes structures.

ZUSAMMENFASSUNG

Durch Atmung einer Mischung während 5–10 Minuten von 7,6 bzw 8,6 O_2 in Stickstoff hat der Verfasser folgende Tests untersucht akustische Raumsensation durch Töne gleicher Intensität aber ungleicher Frequenz dychotischer Test vokale Tests mit Distorsion akzellerierter und unterbrochener Stimme Bedeutende Änderungen wurden für die Raumsensation verursacht weniger für die akzellerierte und unterbrochene Stimme Die Sauerstoffverminderung verursacht funktionelle Veränderungen besonders für die Prozesse welche polysynaptische Wege und scharfe neurale Integration gebrauchen

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DISCUSSION

L. Holmgren (1) Could this explain the well-known fact that individuals with presbyacusis have increased hearing difficulties in an atmosphere which gives hypoxia, as exists in the presence of intense smoking, and consequently an over load of carbon oxide? (2) Has the author made correlations with other semi psychological tests such as typewriting, where the percentage of mistakes increases under the influence of hypoxia? (3) Are the changes of the speech-audiogram entirely due to the influence of hypoxia in the subcortical region or also in the inner ear?

J G Hall Mr Menzio said that the results of asphyxia may be cortical or subcortical. We have some evidence that the greatest damage is in the cochlear nuclei. Croigie has counted the number of capillaries in the auditory system, and found the cochlear nuclei, especially the dorsal cochlear nucleus, to be among

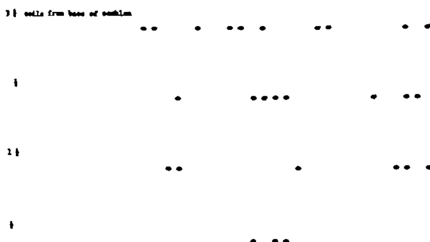


Fig. 1 Cochleogram from guinea pig sacrificed 1 week f ll wing irradiation. Ope circles represent pparently normal cells, solid black circles represent degenerated cells. Left side irradiated with 6000 R.

Dose rate at the site of the inner ear was 200 R/min The X-ray dose was delivered to the left half of the skull through a rectangular opening (20 mm × 17 mm) in a leaden sheet (10.5 mm) which also shielded the rest of the animal. The right inner ear served as control. During irradiation the animal was placed in a cage which did not permit movements of the head Narcosis was not given. The doses applied were 1000 R, 2000 R, 4000 R, 6000 R, and 7000 R. The animals were sacrificed 3 hours, 6 hours, 18 hours and 1 week after the irradiation Investigations were performed by phase contrast microscopy ordinary light microscopy and electron microscopy

The inner ear was fixed in chilled eronal buffered 1.5% osmiumtetroxide solution and kept refrigerated at 40 C for 1–2 hours. For examination by phase contrast microscopy samples of each of the four coils of the organ of Corti (Engström *et al* 1966) and the sensory areas of the cristae and the maculae (Lindeman, 1967) were dissected and mounted in glycerine. By this method a bird s-eye view of a large number of sensory and supporting cells may be obtained. By focussing the field of vision, an optical section ing of the specimen may be performed, permitting a study of different parts of the sensory and supporting cells. Ordinary light microscopy was per formed on stained sections of parafin- or araldite-embedded material. For the electron microscopy the cristae and the maculae and parts from each coil of the organ of Corti were dissected and embedded in epon or araldite, sectioned on an LKB ultramicrotome and mounted on copper grids with formvar coating. The sections were then stained with uranyl acetate and lead citrate (Reynolds, 1963)

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EARLY DEGENERATIVE CHANGES IN THE INNER EAR SENSORY CELLS OF THE GUINEA PIG FOLLOWING LOCAL X RAY IRRADIATION

A *Preliminary Report*

Finn Ø Winther

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X ray irradiation in single doses was applied to the inner ear of guinea pigs. The organ of Corti and the vestibular end organs were examined by light microscopy using the surface specimen technique proposed by Engström A characteristic pattern of degeneration was found. The outer hair cells of the two basal coils of the cochlea were extensively degenerated whereas the outer hair cells of the two apical coils and the inner hair cells were intact In the vestibular end organ sensory cells of the periphery of the cristae ampullares and the maculae utriculi and sacculi proved to be more vulnerable than those of the central parts. A preliminary report of an electronmicroscopic study of the early degenerative changes is given

The effect of ionizing radiation on the inner ear has previously been studied by histological and functional methods (for references see Kelemen, 1963) The histological examination of the irradiated inner ears has, however been performed on stained sections with ordinary light microscopy The results of these studies have been controversial and a re-investigation of the problem with new methods was therefore thought to be of interest In the present investigation, the surface specimen technique recently applied by Engström (Engström *et al.*, 1966 Lindeman, 1967) was adapted and in addition light and electron microscopy on stained sections was used in order to get an answer to the following questions

1 Does irradiation of the inner ear with X rays cause any damage to the sensory cells?

2 *Do X rays bring about any characteristic pattern of damage?*

3 Which are the first signs of damage and how soon after the irradiation are these seen?

MATERIAL AND METHODS

Guinea pigs were used as experimental animals. The source of radiation was a Siemens Stabilipan roentgen apparatus operated at 200 kV 12 mA, and equipped with a Thoraeus filter II (0.8 mm Sn 0.25 mm Cu 1 mm Al)

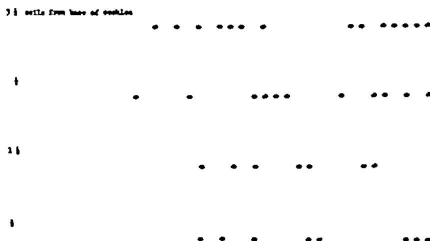


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FIG. 2. Surface specimen from the organ of Corti of the guinea pig, 1st coil from the base of the cochlea. The animal was irradiated with 6000 R and sacrificed 1 week later. Phase contrast micrograph. Focal plane at the level of the cuticular plates. *IHC*, inner hair cells, 1, 2, and 3. First, second and third row of outer hair cells. Note that the inner hair cells are intact, and that most of the outer hair cells are degenerated, showing the typical "collapse" figure (*arrow*). × 700, obj 100, oc 8.

FIG. 3. Surface specimen from the periphery of the crista ampullaris lateralis of guinea pig irradiated with 7000 R and sacrificed 1 week following irradiation. Phase contrast micrograph. Focal plane at the level of the cuticular plates. The degenerated sensory cells show the typical "collapse" figure (*arrow*). × 700, obj 100, oc 8.

RESULTS

The first question (does irradiation of the inner ear with X rays cause any damage to the sensory cells?) could be clearly answered with yes. When doses of 6000 R and 7000 R were applied degenerated sensory cells in a number exceeding that on the not irradiated side was evident. Also the second question (do X rays bring about any characteristic pattern of damage?) could be answered with yes. Phase contrast microscopy of the organ of Corti showed that extensive degeneration was found in the outer hair cells of the basal coil and usually also in the outer hair cells of the second coil from the base of the cochleae (Figs. 1 and 2). The outer hair cells of the two apical coils and the inner hair cells were normal. In the vestibular sensory epithelium the periphery of the cristae and the maculae showed the greatest number of degenerated cells while the number of degenerated cells in the central areas were clearly smaller (Fig. 3). No clear difference in the number of degenerated sensory cells between the cristae and the maculae was found although there was a tendency toward a smaller number of degenerated sensory cells in the maculae sacculi than in the maculae utriculi and the cristae ampullares. The question whether the degenerated vestibular sensory cells were of the type I or II could not be revealed with certainty by light microscopy. By electron microscopy, however, degenerated sensory cells both of type I and II were found.

By phase contrast microscopy of animals irradiated with 7000 R and sacri

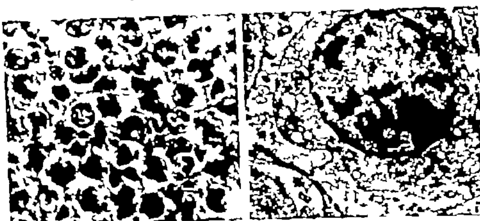


Fig. 4. S rface specimen from the periphery f the crista ampullaris lateralis f guinea pig irradiated with 7000 R d sacrificed 3 hours following irradiation. Phase contrast micrograph. Focal plane t the lev l f the sensory cell uclei. Arrows poi t to nclei with coarse granulation. 800, obj 100, oc. 10.

Fig. 5. Sensory cell type II from the crista mpullaris laterali f guinea pig irradiated ith 7000 R nd sacrificed 3 hours f llowing irradiation. Cl mping of the hromati of the nucleus is seen and merou anu les of moderat ize are present in the cyto-plasm. 7,500.

ficed 3 hours following the irradiation an answer was given to the third question (what are the first signs of damage and how soon after the irradiation are these seen?) The nuclei of the outer hair cells of the basal coil f the organ of Corti were coarsely granulated but had retained their shape and size In animals sacrificed 6 hours following irradiation many of the nuclei of the basal coils were pycnotic. In these cells changes which ranged from a slight distortion of the normal W-pattern of the sensory hairs to incipient formation of a spider like degeneration figure in the cuticular region was found Eighteen hours following irradiation completely degenerated outer hair cells were found in the two basal coils.

Phase contrast microscopy f the vestibular epithelium of animals irradiated with 7000 R showed that 3 hours following the irradiation some of the sensory cell of the periphery had a coarsely granulated nucleus (Fig. 4) In these cell the sensory hair bundles were usually distorted and often a spiderlike degeneration figure in the cuticular plane was seen. At 6 hours following irradiation all stages of degeneration from a slight coarse granula tion f the nucleu to complet ly degenerated sensory cells were observed Eighteen hours following irradiation many totally degenerated sensory cells were found in the periphery of the cristae and the maculae

The early degenerative hanges were lso studied by electron microscopy Only a small number of animals have so far been examined, and the results presented are therefore preliminary

In its broad features the mod of degenerati n seemed to be the same in the sensory cell of the organ of Corti and the sensory cells of the vestibular



Fig 2 Surface pecimen from th organ of Corti of th guinea pig, 1 / cells from the ba e of the cochlea Th animal was irradiated with 6000 R nd sacrifi ed 1 week later Phase contrast micrograph. Foc l plane at th level f th cuticular plates. IHC, I ner hair cells, 1 2, and 3 First, second and third row of outer hair cells. Note that the inner hair cells are intact and that m st of th uter hair cells re degenerated, showing the typical "collapse figure (*arrow*) 700, bj 100, oc 8.

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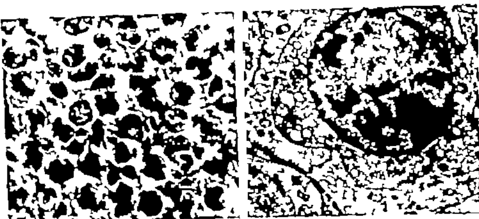


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In the organ of Corti the outer hair cells of the basal coils were found to be most vulnerable to X-ray irradiation It is interesting to note that the same cell group also was affected first when guinea pigs were given large doses of antibiotics of the streptomyces group (Hawkins & Engström, 1963 Kohonen, 1965) In the vestibular sensory epithelium however the greatest sensitivity to X-ray irradiation was in the periphery of the cristae and the maculae. Here however the largest damage caused by antibiotics of the streptomyces group is found in the central regions (Lindeman, 1967) These variations in vulnerability of various cells are not readily explicable but may possibly be related to differences in morphology and biochemistry

The first effect of radiation on living matter is physical in that it affects atoms and molecules irrespective of their arrangement in living structures, resulting in splitting of the molecules into radicals and ions. The chemical balance of the cells is thus upset. A possible explanation of the sequence of events observed by electron microscopy is that the DNA/RNA system is affected resulting in vacuolisation of the endoplasmatic reticulum due to an abnormal prothein synthesis.

Referring to this it is interesting that instillation into the guinea pig bulla tympanicum of streptomycin, which interferes with the prothein synthesis (Gorini, 1966) gives a similar electron microscopic picture (Spoendlin, 1966) as that observed following X ray irradiation.

RÉSUMÉ

L'irradiation par des rayons X fut appliquée à dose isolée da s l'oreille interne du cobay L organe de Corti et l'extrémité des organes vestibulaires furent examinés da l'éclairag microscop que en utilisant la tech ique des spécimens de surfac proposée pa Engström. Un genre de dégénérati n caractéristique fut trou é Les cellules ciliées externes des deux tours de spin de la cochlée situées à la base étaient degénérées d façon pron ncée, tandis que les cellules ciliées externes appartenant a deux tours apicaux et les cellules ciliées internes étaient intactes. Dans l'organe terminal vestibulaire, les cellules sensori lles de la périphéri du cristae amp llares, du maculae utriculi et du sacculi, démontrent qu elles sont plus vulnérables que celles de la partie centrale. Un reportage préli min ire aussi été f it, concerna t l'étude au microscope électronique des altérations dégénératives précoces.

ZUSAMMENFASSUNG

Die Wirkung einer Einzeldosis von Röntgenstrahlen ai di Sinnes-Zellen des inneren Ohres von Meerschwei chen wurde lichtmikroskopisch an Präparaten untersucht, di ach der Methode von Engström hergestellt waren. E konnt ei harakteristisches Degenerationsmuster festgest llt werden. Di äusseren Haarzellen der beiden basalen Schneckenwindungen zeigten ausgedeh te degene-

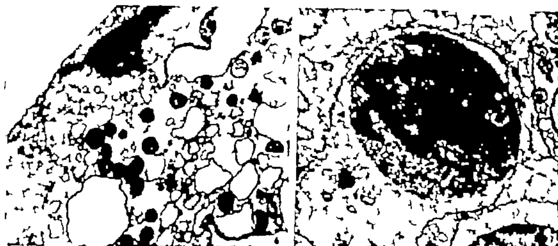


FIG 6 Subcuticular region of an outer hair cell of the organ of Corti / a coll from the base of a guinea pig cochlea Th animal wa irradiated with 7000 R and sacrificed 3 hours later Marked vacuolisation of th cytoplasm The cuticular pl t is intact × 12,500

FIG 7 Sensory cell type II from the crista ampullaris lat rali of a guinea pig irradiated with 7000 R and sacrificed 6 hours l ter The nucleu is deformed and the chromatin clodded in dense clumps. The cytopl sm is highly lectrondense A f w v cu les (*arrows*) and ribosome like granules are vi ible in the cyt plasm. × 10,000

epithelium As was assumed from light microscopy the first sign of damage to the cell was a clumping of the chromatin granules of the nucleus. In many cells this was found without any changes of the cytoplasmic or ganelles In other cells where the nuclear changes were more pronounced, there were cytoplasmic vacuoles, often of considerable size and with the surface lined with ribosomelike granules (Figs. 5 and 6) At this stage swollen mitochondria, often with disrupted cristae vacuole formation, and myelin figures could be found side by side with apparently normal mito-chondriae A special feature of the vestibular sensory cells was expulsion of portions of the cytoplasm on the endolymphatic surface Before the ulti mate resorption of the degenerated sensory cell the cytoplasm and nucleus underwent pycnosis (Fig. 7) Except for a few vacoules and ribosome-like granules, details could not be recognized in the highly electrodense cell This stage of degeneration was reached within 6 hours from the completion of irradiation.

DISCUSSION

Cell degeneration following exposure to X rays may be caused by a direct action in the degenerating cell, or it may be caused by interference with the blood supply A third possibility is a combination of these factors During the preparation of the temporal bones no gross damage to the vessels result ing in hemorrhage or exudate was noted Neither did preliminary light and electron microscopic study of the vessels reveal any damage The functional state of the vessels however cannot be judged from histological specimens alone (Kreyberg 1928)

A characteristic pattern of sensory cell degeneration was found both in the organ of Corti and the vestibular epithelium

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RESUME

L'irradiation par des rayons X fut appliquée à dose isolée dan l'oreille interne du cobaye. L organe de Corti et l'extrémité des organes estibulaires furent examinés dan l'éclairage microscopique en utilisant la tech ique des spécimens de surf ce proposée par Engström. U genre d dégénération caractéristique fut trouvé Les cellules ciliées externes des deux tours de spir de la cochlée sit ées à la base étaient dégénérées de f çon prononcée, ta dis que les cellules ciliées externes pparten nt ux deux tours pical et les cellules ciliées internes étaient intactes. Dans l' rgane terminal vestibulaire, les cellules sensori lles de la périphérie du cristae ampullares, du maculae utriculi et du sacculi, démontrent qu elles sont plus vulnérables qu celles de la partie centrale. Un reportage prélim naire aussi été f it, concernant l'étude u microscope électronique des altéra t ons dégénératives précoces.

ZUSAMMENFASSUNG

Die Wirkung einer Einz ldosi on Rontgenstrahlen uf die Si nes-Zellen des neren Ohres von Meerschw i chen wurd ll htmikrosk pisch an Präparate tersucht di sch der Methode on Engström hergestellt waren. E konnte ei charakteristisches Degenerat onsmuster festgest llt werden. Die äusseren Haarzellen der beiden basalen Schneckenwindungen zeigten usged hnt degen

rative Veränderungen während die entsprechenden Zellen der beiden apikalen Windungen und die inneren Haarzellen normal waren In dem vestibularen Teil des inneren Ohres zeigten die Sinnes-Zellen in der Peripherie der Cristae ampullares und der Maculae utriculi und sacculi grössere Vulnerabilität als die entsprechenden Zellen in den zentralen Regionen Ein Präliminar Rapport über das Elektronmikroskop-Studium der frühen degenerativen Veränderungen ist gegeben.

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OTOSCLEROSIS AND OSTEOGENESIS IMPERFECTA

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The possibility of a common aetiology of otosclerosis and osteogenesis imperfecta is discussed. The histopathological differences in two temporal bones, one with osteogenesis imperfecta congenita and one with co-existing osteogenesis imperfecta tarda and otosclerosis, are emphasized On the basis of the method currently used it cannot be confirmed, that otosclerosis and osteogenesis imperfecta have a common aetiology

In the effort to find the early changes in otosclerosis and to view otosclerosis as a manifestation of generalized disease attention has been focussed, since the beginning of this century on well-known bony dystrophies, such as osteitis fibrosa or Recklinghausen s disease, osteitis deformans or Paget s diseases, and osteogenesis imperfecta.

Clinically and aetiologically these diseases differ but histologically they are similar in many respects.

In cases of incipient otosclerosis histological examination reveals osteoclastic destruction of the bony tissue in the labyrinthine capsule followed by new formation of abnormal bony tissue. The latter presents itself as tissue of a mosaic pattern with large medullary cavities and cellular highly vascularized bony tissue poor in fibrils. However a finding of destruction and new formation of bony tissue at the same time is typical of all vital bony changes.

A possible relationship between otosclerosis and osteogenesis imperfecta has been a subject of quite particular interest

Osteogenesis imperfecta is a relatively uncommon, inherited, generalized connective-tissue disease The characteristic syndrome of blue sclerae brittle bones with a tendency to fractures, and hearing loss of a conductive type is generally related to the names of van der Hove and de Kleijn who were the first to suggest, in 1918, that the hearing loss was due to otosclerosis. Ruttin (1922) Nager (1922) and Gimplinger (1926) are said to have been the first to claim that they could confirm this histologically

The heredity is believed to be by a dominant Mendelian trait (Seedorf 1949)

Weber in 1930, found in the callus in a healing fracture in a patient with osteogenesis imperfecta, bony changes highly reminiscent of the so-called blue mantles in typical otosclerotic foci. When considering also the mode of

rative Veränderungen während die entsprechenden Zellen der beiden apikalen Windungen und die inneren Haarzellen normal waren. In dem vestibularen Teil des inneren Ohres zeigten die Sinnes-Zellen in der Peripherie der *Cristae ampullares und der Maculae utriculi und sacculi* grössere Vulnerabilität als die entsprechenden Zellen in den zentralen Regionen. Ein Präliminar-Rapport über das Elektronmikroskop-Studium der frühen degenerativen Veränderungen ist gegeben.

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Fig. 3. Fissula ant fenestram, ...ula ligament, nd stapes X otosclerosi or fixation of the st pes (case 1)

Fig. 4. Inner layer of the labyri thine capsule round the superior semicircula canal. A narrow ndosteal layer nd w ll-m rked endochondral la er X marrow medull ry ca ities nd cartilag remnant in the immature bony tissue (case 1)

centrally around the cochlear lumen and a somewhat thicker more continuous layer peripherally Between these two layers there were bone marrow spaces. The ground substance consisted of few irregularly arranged fibres. Osteocytes were of irregular shape and arrangement The amount of cartil lagin us tissue was relatively increased (Fig. 2)

The periosteal capsule was poorly developed, consisting of a cellular web-like bony tissue of a somewhat coarser fibrillar structure than the endochondral layer In several places the cells were large with vesicular nuclei.

However there was a gradual transition between the peripheral part of the endochondral layer and the periosteal layer the latter showing a denser fibrillar structure

The vestibula part of the labyrinthine capsule showed approximately the same appearance The endosteal layer was poorly developed and the endochondral layer here too, divided into a less continuous layer centrally and a thicker continuous layer peripherally—with large cartilaginous islets, especially around the fissula ante fenestram (Fig. 3) In this part too the periosteal layer was thin.

Similar changes were f und in the capsule surrounding the semicircula canal (Fig. 4) with a faint, but normal developed endosteal layer The endochondral layer was of the same structure as in other areas of the labyrinthine capsule

Case 2

Both temporal bones from a 77 year-old man Clinical diagnosis Osteogenesis imperfect tarda

In the labyrinthine capsule n both sides severe abnormalities, and two types of abnormal tissue could be distinguished (Fig. 5)

Primarily pronounced otosclerotic changes, about threequarters of the



FIG. 1 Labyrinthine capsul with congenital osteogenesis imperfecta Cochlea, part of the vestibule and the footplate of th stapes (case 1)

FIG. 2 Basal turn of cochlea. Between the stria vascularis and the immature bo e m trix a well marked lay r of chondrocytes (case 1)

inheritance and the common clinical co-existence of the two diseases, this finding pointed to a common genesis.

In recent years Simson Hall and his associates (1961 1962) as well as Wullstein (1960) have studied the relationship between osteogenesis im perfecta and otosclerosis They arrived at the conclusion that the two diseases are due to a common genetic anomaly in the osteoblasts, otosclerosis being interpretable as a localized form of osteogenesis imperfecta, differing from it only in degree, extent and localization

In contradistinction Altmann *et al* (1962, 1967) in particular have not in their own studies or in the literature on the subject been able to find changes suggesting otosclerosis in temporal bones affected with osteogenesis imperfecta Accordingly they do not feel there is histological or histochemical evidence of a common aetiology but the frequent co-existence of the two diseases might perhaps be due to osteogenesis imperfecta predisposing to the development of otosclerosis by way of a still unknown chemical or ultramicroscopic structural abnormality

In a study of the temporal bones in our laboratory we have found two cases of osteogenesis imperfecta

Since one of these cases also exhibited otosclerotic changes, we felt that our findings were worthy of publication

Report of Cases

Case 1

Both temporal bones from a newborn infant with osteogenesis imperfecta.

In the bony cochlear capsule (Fig 1) the endosteal layer was very thin but normal A continuous, darker line separated this layer from the endochondral layer which was well-developed and made up of a delicate skein like, immature, and irregular bony tissue with ample newformation of bone The endochondral layer formed a thin more or less continuous layer



Fig. 3. F ia nt fenestram, la fig ment, d st pes A otosclerosi fissula of the st pes (se 1)

Fig. 4. I ne layer of the labyri thine caps le round th perior semicircular nal. A narrow ndosteal layer nd a w ll-marked endochondral l y Numerous med ll ry ca ities nd cartilag remnant f the immat re bo y tissue (case 1)

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Similar changes were found in the capsule surrounding the semicircular canal (Fig. 4) with a f int, l ut normal developed endosteal layer. The endochondral layer was of the same structure as in other areas of the labyrinthine capsule

Case 2

Both temporal bones from a 77 year-old man. Clinical diagnosis Osteogenesis imperfecta tarda.

In the labyrinthine capsule on both sides severe al normalities, and two types of abnormal tis ue could be distinguished (Fig. 5)

Primarily pronounced otoscler tic changes, about threequarters of the

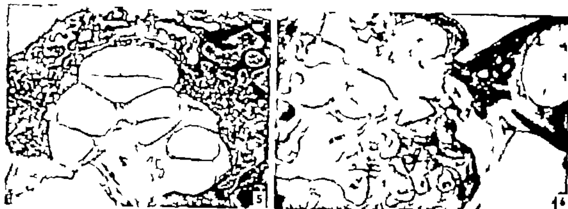


FIG. 5 Part of the labyrinthine capsule with otosclerosis and osteogenesis imperfecta tarda (case 2)

FIG 6 Oval window Th otosclerosis has encroa hed upon th footplat of th stapes. Adj cent to the vestibule also osteogenesis imperfecta tarda (case 2)



FIG 7 Osteogenesis imperfecta tarda in close rel ti n to the cochlea In th right ide f th picture otosclerotic ti ue (case 2)

FIG. 8 Sharp borderline between otosclerotic tissue on th left and osteogenesis imper fecta tarda on the right ide of th picture (case 2)

total labyrinthine capsule having been converted into tissue of otosclerotic type In most sites the process was highly active there being ample vascu larized connective tissue with osteoclastic as well as osteoblastic activity in the marrow space In other sites the activity was less marked in a few sites the process was outburnt leaving empty marrow spaces and bony tissue of low vitality showing only scattered degenerated osteocytes In the oval window the otosclerosis on both sides had encroached upon the foot plate and entailed ankylosis of the stapes (Fig 6)

Otosclerotic changes were most pronounced in the cochlear part of the labyrinth, gradually losing themselves in the vestibular part

The other type of tissue changes in the labyrinthine capsule affected the normal bony tissue In close relation especially *to the lateral border of the* basal cochlear turn (Fig 5) but also around the utricle and saccule there was bony tissue showing the changes of osteogenesis imperfecta *tarda*

In the bony cochlear capsule the endosteal bony layer was thin but of normal appearance.

In large areas the endochondral layer (Fig. 7) was built up of very dense, cellular bony tissue, where the normal lamellar bony tissue was replaced by homogeneous bony tissue with cartilage remnants. The borderlines between this tissue, the normal bony tissue, and the otosclerotic tissue were sharp (Fig 8)

Thus, the normal bony tissue showed quantitative changes, in the form of appreciable increase in the relative amount of connective tissue.

DISCUSSION

As pointed out by Altmann & Kornfeld (1967) it has not yet been proved that otosclerosis and osteogenesis imperfecta have a common aetiology in spite of histological similarities—viz. the immature bone matrix which in congenital osteogenesis imperfecta is highly reminiscent of the blue mantles of otosclerosis.

Our findings support Altmann & Kornfeld s view that where otosclerosis and osteogenesis imperfecta co-exist the two conditions may be clearly distinguished histologically In our first case the histological finding characteristic of congenital osteogenesis imperfecta, was an important component in the endochondral layer of the labyrinthine capsule. In the second case there was a distinct histological difference between the otosclerotic tissue and the tissue affected with osteogenesis imperfecta. It cannot be decided whether the otosclerotic process was due to the generalize skeletal disease or whether the simultaneous presence of otosclerosis and osteogenesis imperfecta was a chance coincidence However there is no histological resemblance between the diffuse skeletal changes in osteogenesis imperfecta and the changes in the otosclerotic focus.

Clinically the two diseases often co-exist. Fowler s study (1949) for instance supports the view of Wullstein-Hall who found that 69% of patients with fixation of the stapes have abnormally blue sclerae. Both diseases are hereditary and more common in women than in men. Similarly 40–60% of patients with osteogenesis imperfecta have fixation of the stapes and blue sclerae

Biochemical and histochemical studies (Chevance *et al* 1962 Chevance, 1964 Arslan & Ricci, 1963) have thrown new light upon the relationship between the two diseases. As already mentioned, Simson Hall *et al* believe that both are due to a common genetic anomaly in the osteoblast, resulting in the immature bony matrix seen particularly in osteogenesis imperfecta Chevance s studies reveal that the alteration in the enzymic activity in the osteocyt is the primary factor in the development of an otosclerotic focus.

Through 3 years Chevance (1965) studied about 2500 operative specimens from patients with fixation of the stapes. Of these, 500 were studied histologically as well as histochemically Only eight of the specimens were

derived from patients with osteogenesis imperfecta. As decisive differences Chevance emphasized that the histochemical studies of osteogenesis imperfecta did not reveal –SH groups and that osteoclasts and osteoblasts were seldom seen at the oval window Furthermore Chevance never observed blue mantles in osteogenesis imperfecta whereas "microfractures" were common

Arslan & Ricci (1963) consider otosclerosis as a local manifestation of a generalized connective tissue disease involving alterations in the ground substance of the supporting tissue Vyslonzil's (1956) Bentzen's (1961) and Stadil's (1961) findings of histological changes in the connective tissue of skin biopsies from patients with otosclerosis and from patients with osteogenesis imperfecta point into the same direction Soifer *et al* (1965) have found an increased lactate dehydrogenase activity in the venous wall from otosclerotics compared with normals, and this too indicates a generalized connective tissue disorder Altmann & Kornfeld (1967) believe that the reason why uniform histochemical changes, in the form of an increased quantity of acid mucopolysaccharides and fewer fibrils, are found in the two diseases, is the morphological similarity between blue mantles of otosclerosis and the immature bone matrix of osteogenesis imperfecta.

In other words, opinions are divided and as yet it is not possible on the basis of the studies performed so far to draw any conclusion about a common aetiology of otosclerosis and osteogenesis imperfecta.

RÉSUMÉ

La possibilité d une aetiologie commune entre otospongiose et osteogenesis imperfecta est discutée. Les différences de la histopathologie dans deux os temporales, un avec osteogenesis imperfecta congenita et un avec otospongiose et osteogenesis imperfecta tarda en même temps, sont soulignées. Ce n est pas possible avec les methodes de recherche qui sont employées aujord'hui, de confirmer une aetiologie commune entre otospongiose et osteogenesis imperfecta.

ZUSAMMENFASSUNG

Die Möglichkeit dass Otosklerosis und Osteogenesis Imperfecta eine gemeinsame Äthiologie hat, wird diskutiert Die histopathologischen Verschiedenheiten in zwei Schläfenbeinen, ein mit Osteogenesis Imperfecta Congenita und ein mit gleichzeitiger Osteogenesis Imperfecta Tarda und Otosklerosis werden betont. Es ist heute mit den neuen Untersuchungsmetoden nicht möglich eine gemeinsame Äthiologie von Otosklerosis und Osteogenesis Imperfecta zu bestätigen.

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DISCUSSION

A Laskiewic The middle layer of the labyri thine capsule i from the earliest day of life only partially replaced by en hondral bone s. c. globuli ossei, while the major portion is replaced by bone *marrow* which i then compacted by pposition f endosteal convoluted bon a d the mesenchymal bon forming cells are able t form bo e directl. In a number of cases it forms a secondary hondroid type of cart lage which may be replaced by enchondral globuli ossei. Osteoclastic resorbtion and lacunar erosions with accumulations of

bone forming mesenchymal cells within the eroded lacunar areas are still present. Otosclerotic bone within its areas is a new bone formation within fibrous tissue, while the s.v invasive bone spreads like a wave into the old capsular tissue with confluence of the perivascular bone areas. Blue mantles represent an attempt by the uninvolved portion of the capsule to repolymerize mucopolysaccharide matrix which loses its mineral by a process of halisteresis rapidly along physiological channels as remodeling rate about the vessels with increased mineralisation osteoclastic resorbtion and accumulation of bone forming mesenchymal cells for the extensive degeneration in adjoining areas. As for the factors being responsible for the process in question it may be quoted that hereditary constitutional factors, thermal, inflammatory and mechanical (acoustical) ones, and irradiation have been suggested by Kristensen to initiate a preparedness in developing the otosclerotic process by inducing activation of dormant embryonic or a presence of repair upon a microscopic focus in aseptic necrotic area caused by irradiation

H C Andersen Mr Bretlau shows microscopy findings in three patients suffering from osteogenesis and otosclerosis. In one typical otosclerotic changes were found in the stapes. In two other cases there was no sign of otosclerosis, but changes resembling osteogenesis imperfecta. It is concluded that conductive deafness in osteogenesis imperfecta can be produced by otosclerosis or by osteogenesis in itself

J G Hall First I think it is appropriate to recall the findings which our former professor and member of this Collegium Odd Opheim, published in the Acta no 65 this year During the period 1960–65 Opheim in five cases of osteog imp found epithelium luneo cavities in the capitulium stapedii and structurally alteren crurae with a fibrous degeneration

Mr Röhrt and myself in the same number of the Acta reported a case in which histological examination of the stapes was performed. No otosclerotic foci were found the stapedial joints were normal, but the crurae were thin, frayed, in some places thread like with only a fibrous connection to the footplate

Today we hear *that histological changes of this type are found in connection* with otosclerotic foci just like others have reported before I think it is reasonable to conclude that we do not have enough support for the presumption of a basic and common ethiology in these diseases. We are still at a loss concerning the explanation of these coincidences.

Balslev Jörgensen (Reply) to Mr *Laskiewicz* We want to point out that in our studies of temporal bones we have found the so-called blue mantles" in normal bones as well as in different pathological conditions. Consequently we do not consider blue mantles as a sign of latent or incipient otosclerosis.

To Mr *Andersen* Your slides seem to support our view that otosclerosis and osteogenesis imperfecta are two clearly distinguishable conditions from the histological point of view

To Mr *Hall* Your comments point in the same direction. We apologise that we forgot to mention Professor Oppheim s important paper which appeared recently in the Acta.

Acta oto-laryngologica 67 277 280 (1969)

QUELQUES RÉSULTATS DES EXAMENS OTOLOGIQUES ET AUDIOLOGIQUES DES INDIENS SUD-AMÉRICAINS

J M Tato et J M Tato

Buenos Aires Argentine

On a e aminé oto- et udi logiquement 11 000 individus, dans différentes zo es du Pérou, de la Bolivie et du Paraguay appartenant au groupe d'Indiens, Andidos ou Pampidos, d t 6500 de race pure u presque et 5000 métis. Quant à l otosclérose, chez le groupe Indien pur on a trouvé 2 cas unilatéraux probables, ce qui donn rait un indice de 0,03% Chez les métis 0,3%

Le Núcleo Latinoamericano para el estudio de la audición y equilibrio » a décidé de promouvoir une étude sur l audition de la population indienne de l'Amérique Latine

Une équipe dirigée par J M Tato (p) a été constituée par les docteurs Jaime Flores, comme directeur-assistant, J M Tato (f) Aníbal Arabel, Luis Vinatea et les audiologistes E. Sarrail, M Lauberer et E. T de Staria avec la collaboration temporaire selon les lieux, des docteurs R. Porcel, A Bustillo, Alfredo Padilla en Bolivie D Tejada et G. del Carpio au Pérou Drs. G Santos et R. de la Torre au Paraguay

L'investigation commença en 1961 à Salta, Oran, Argentine, dans le N O près de la frontière bolivienne.

A partir de 1963 elle se poursuivit au Pérou avec l'appui économique d un fonds N.B.03 006.01-03, de l Institut National de la Santé, du Département de la Santé de l'Education et de l'Hygiène des Etats-Unis d Amérique. Tenant compte de l opinion des autorités de la Santé Publique et des Instituts Indigènes des différents pays, les endroits furent choisis en fonction des possibilités des conditions locales et de l'acceptation par la population d'être xaminée

En voici la liste

Année	*Zone et pays*	*Nomb e d'examens*
1961	Oran, Salta, Argentine	591
1963	Yunguyo, Pérou	1 338
1963	Pun et Lauca, Pérou	93
1963	Tacna, Pérou	710
1964	Lima, Pérou	1 416
196.	Hacienda Vicos, Pérou	600
1965	Hu raz, Pérou	950

1965	Asunción, Paraguay	100
1965	Filadelfia, Paraguay	1 818
1966	Siglo XX et Catavie, Bolivie	3 039
1966	Irphachico Bolivie	1 102
	Total	*11 757*

Du total 1378 furent examinés otologiquement, l examen fonctionnel étant limité aux diapasons, et chez tous les autres (10 379) une audiométrie liminaire aérienne et osseuse fut pratiquée

En cas de suspicion d otosclérose l éxamen fonctionnel fut complété par *les épreuves de Gellé, Runge, l indice de Sullivan* et dans les derniers cas par la mesure de l impédance absolue et relative

Les objectifs de l étude furent multiples l un d eux a été d établir l existence de l otosclérose et si oui, dans quel pourcentage Des questions connexes ont été également étudiées.

La population indigène pure (du moins au plus grand dégré) a été de 6 163 pour 4 340 métis (*blancs et indiens*) *Ces chiffres résultent* des études statistiques faites par le Département de la Santé Publique de la République Argentine au moyen de machines calculatrices et d ordinateurs Les âges limites des personnes examinées étaient de 8 et 85 ans Chez les indigènes, nous avons trouvé deux cas probables d otosclérose unilatérale Chez les métis 5 cas Cela represénte

	% *d olosc.*
Parmi 6163 indiennes pures de 8 à 85 ans	0 03
Parmi 5141 indiennes pures de 20 à 85 ans	0 04
Parmi 4340 métis de 8 à 85 ans	0 11
Parmi 3408 métis de 20 à 85 ans	0 14
Guild parmi 585 temporaux de blancs de 1 à 80 ans	7 25
Guild parmi 518 temporaux de blancs de 6 à 80 ans	8,30
Sercer parmi 200 crânes de blancs	1,25
Cliniquement parmi la population blanche de 0 50 à 1	

Rosen n a pas rencontré d otosclérose chez les Nègres ni au Sudan ni aux Bermudes S Guild a trouvé dix fois plus de cas d otosclérose dans la population *blanche que dans la population noire de Baltimore* Ainsi il existe une grande différence d apparition de l affection entre les races

Selon Sehcer cette différence serait dûe à la torsion de la pyramide pétreuse et du labyrinthe qui se manifeste dans les variations de l angle sphénoïdal qui est différent selon la race

Ceci nous a incités à le mesurer chez les Indiens, étude effectuée par J M Tato (f) grâce à des radiographies de profil et des tomographies sagittales du crâne dans la population actuelle.

En voici les résultats promediels

8 crânes secs de Pampidos argentins	130 7
7 quechuas vivants du Pérou	135 ,3
16 aymaras vivants du Pérou	134 6

Il a pratiqué des mesures directes de l angle sphéno-occipital ou de Wel ker sur 186 crânes secs provenant d'Indiens de Patagonie et du Nord-Ouest, appartenant au Musée Anthropologique National d Argentine.

Parmi ceux-ci existent des crânes normaux et des crânes déformés selon la tradition.

Les valeurs moyennes furent les suivantes

— Crânes normaux	132 ,3
— *Crânes déformés*	
— tabulaire erect	134 6
— tabulaire erect plan frontal	138 3
— tabulaire erect plan labdoïde	133 6
— tabulaire oblique	142 6

Selon Sercer ces valeurs seraient pour

— las crânes yougoslaves	117
— les crânes japonais	135
— les crânes des anciens péruviens et boliviens	132

Nous devons souligner la coïncidence entre les valeurs angulaires des crânes des Indiens Sud Américains et des Japonais (selon Sercer) et la concordance avec l'hypothèse de Sercer selon laquelle les Indiens ont un angle beaucoup plus ouvert que les blancs.

Les mesures d'impédance absolue et relative chez les Indiens péruviens et boliviens coï cident avec celles obtenues au Danemark, aux U.S.A. et à Buenos Aires (Argentine)

On recontre des différences morphologiques dans la forme du pavillon, parmi lesquelles le prolongement de la racine de l'hélix qui divise la conque en deux parties dans 5% des cas le conduit auditif est droit dans sa paroi antérieure osseuse et dans les crânes préhistoriques du Musée Anthropologique de La Paz (Bolivie) sa paroi antérieure est même concave l'insertion tympanique se fait dans un plan plus oblique vers l avant ce qui explique l'absence ou la modification du triangle lumineux et le raccourcissement apparent de la paroi postérieure du tympan dû à la perspective. Ces modifi cation n nt jamais été décrites à notre connaissance

Dans des publications antérieures nous avons décrit des cas d'otosclérose chez des Indiens prétendu purs en dehors de notre enquête actuelle Nous les a on fait examiner par des anthropologistes qui ont conclu qu'il agi sait san aucun doute de métis.

Des rares cas ont été décrits par des collègues boliviens, mais sans confir mation anthropologique

Dan une des régions (Irpachico, Bolivie) caractérisée par son endogamie

pendant plusieurs siècles où l incidence d une maladie héréditaire devrait être plus élevées nous avons examiné 25 % de l ensemble de la population, soit 1088 sujets sans trouver aucun cas d otosclérose

SUMMARY

A group of 11 000 people belonging to the Andido and Pámpido Indian races were examined otologically and audiologically in different parts of Peru Bolivia and Paraguay About 6500 of these were real Indians and about 5000 were half breeds As regards otosclerosis in the group of pure Indians only two unilateral probable cases were discovered giving therefore an average of 0 03% For the half-breeds it was also 0.3%

ZUSAMMENFASSUNG

Wir untersuchten otologisch und audiologisch 11 000 Indians aus der Gruppe der Andiden oder Pampiden aus verschiedenen Regionen Perus, Boliviens und Paraguays. Davon waren 6500 reinrassig oder beinahe und 5000 Mischlinge Bezüglich Otosklerose fanden wir unter den reinrassigen Individuen 2 wahrscheinlich einseitige Fälle was einen Index von 0 03% ausmacht Unter den Mischlingen betrug er 0,3%

Az néaga 235
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VISUAL REINFORCEMENT AUDIOMETRY

G LIDÉN and A. KANKKUNEN

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Visual reinforcement audiometry is a new technique for testing hearing in very young children. This method is a modification and simplification of co diti ed orient tion reflex audiometry by Suzuki & Ogiba. I stead of usi g th sound localization or eye deviation reflex as a response criterion we are usi g a no directio al technique and accept four different types f responses t th tone stimulation reflexive behaviour in estigatory rientation and spontaneous responses. Hearing levels of normal hearing children of different age groups btai ed with both methods are presented.

The early identification and management of the auditorily handicapped child has been shown to be of paramount importance for the general development of the child as well as for the development of his speech and lan guage Exact knowledge of the auditory function thus is necessary for planning a training program for children with communicative disorders.

The neonatal auditory test procedures described by Ewing & Ewing (1947) Wedenberg (1956) Hardy *et al* (1959) and Downs & Sterritt (1964) have proven ery helpful in the early identification of deafness. From the audiological and educational point of view however these methods are too crude and do not provide us with sufficient information concerning the child's threshold sensitivity In fact, until recently the period from birth up to 2 / or 3 years f age, when play audiometry (Barr 1955) can be used, ha remained baffling with regard to threshold audiometry

Suzuki & Ogiba (1960) introduced conditioned orientation reflex audiometry (CORA) as a way out of these difficulties. According to their report th binaural tone thresholds could successfully be determined with more than 85% of 174 children between 1–3 years of age Their method is simple. The child sits in front of the equipment. On each side of its front panel there is a speaker fed by a pure-tone audiometer Above each speaker is a semitransparant doll which can be illuminated. The distance between the speakers is about 70 cm The eye orientation reflex in response to pure tones i reinforced by illuminating the doll on different sides of the panel and the conditioning i made by pure tone Afte a few trials when the child is conditioned he usually looks immediat ly towards the doll in response t the tone stimulus.

Thi study was supported by the S edish Medical Research Council

We have used Suzuki & Ogiba's method and found it very useful in normal hearing young children but too difficult for many hard of hearing children. Reddell & Calvert (1967) seem to be of the same opinion, according to a recent report. Another disadvantage with their method is that it achieves only a binaural threshold. Therefore we modified the equipment and changed the testing technique. Instead of using the sound localization or eye deviation reflex as a response criterion, we used a nondirectional technique and accepted four different types of responses to the tone stimulation: reflexive behavior, investigatory, orientation and spontaneous responses. These types of response will be discussed below. We have called this modification *visual reinforcement audiometry* (VRA).

PRESENT INVESTIGATION

Purpose

The purpose of our investigation was to answer the following questions concerning visual reinforcement audiometry (VRA).

1. Is VRA a successful technique for eliciting responses to auditory stimulation in normal and hard of hearing preschool children?
2. How does the hearing level in normal preschool children compare with normal hearing for adults when using VRA?
3. How do the responses vary in different children of different age groups when using VRA?
4. How does the efficiency of testing the hearing of preschool children using visual reinforcement audiometry compare with that of conditioned orientation reflex audiometry?
5. How long during a test session can we maintain the interest of the child?

Subjects

Normal hearing children

Two groups of subjects were used in this study. One group consisted of 120 children with normal hearing, while the other contained 935 investigations on children with varying degree of hearing impairment. These two groups shall be described in greater detail. The parents of 148 supposedly healthy children who were visiting the two well baby clinics located closest to the hospital for routine check ups were asked to participate in the hearing test. The children were selected at random by choosing every third child belonging to the following age groups: 3 months to 1 year, 1 to 2 years, 2 to 3 years, 3 to 4 years, 4 to 5 years or 5 to 6 years. Clinical examinations revealed 17 children with some middle ear involvement, five with sensorineural hearing loss and six with mental retardation. These 28 children were excluded from this study. The remaining 120 children were distributed evenly in the abovementioned six age groups.

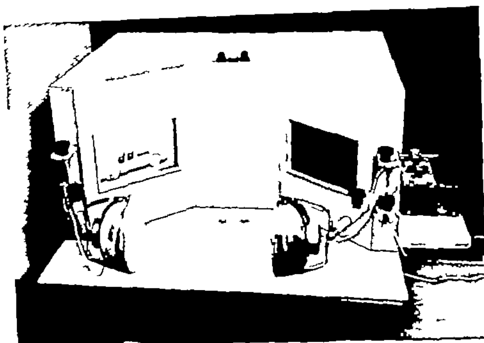


Fig. 1 Anterior view of the visual reinforcement (VR) audiometer

Hard-of-hearing children

Since 1964 visual reinforcement audiometry has been used routinely in our laboratory. In order to answer some of above mentioned questions we have analyzed our records on children with suspected or verified hearing loss up to the end of 1966. Altogether 935 investigations were performed.

Equipment

Instead of using illuminating dolls as visual reinforcement for the orientation eye reflex, we alternately project slides on two frosted glass windows located on either side of the curved front panel of the box shaped apparatus (Fig. 1). The slides are specially selected by our preschool audio-educators to maintain the interest of the child. The switching of the slides from right to left and vice versa is arranged electronically with the help of mirrors (Fig. 2). The switch board is placed on the audiometer. The loudspeakers are mounted on two separate movable and adjustable arms making it possible to present the tones with an azimuth of 90 degrees on each side. The audiometer provides the following test frequencies: 0.25, 0.5, 1, 2, 3 and 4 kHz. In this investigation the tones have been presented steady but later we also included frequency modulation (warble tone). The intensity range is −10 dB to 110 dB regulatory in 5 dB steps. The maximum hearing level is 90 dB for 250 Hz, 100 dB for 500 Hz and 110 for the others. The audiometer with the loudspeaker was calibrated in an anechoic room on

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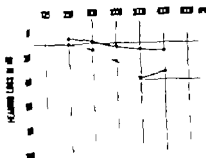


FIG. 3 ( ) The head shadow effect measured artificial head in the sound field f the VR-a diameter The so nd lev l recorded from far ea (*solid line*) is plotted relative t that of near ea (0 dB) (*b*) The "head shadow effect with ea pl g i f ear The sound lev l recorded from fa ear (*dotted line*) is pl tted relativ to that of ar ear (0 dB)

greater deviation from this pattern should arouse suspicion of partial or complete monaural hearing loss. According to Nordlund (1962) the head shadow may reduce the intensity of the stimulus in an anechoic room in the far ear by about 5 dB for the frequencies 250 and 500 Hz and between 10 and 25 dB for 1000–4000 Hz. In other words if we tested the threshold sensitivity on the left side of a child who was deaf on that side we could expect to get a considerable drop of the tone threshold only for the higher frequencies.

In order to find out more about the head shadow effect in the actual testing situation we used an artificial head as described by Nordlund & Lidén (1963) as a substitute for the child's head. In Fig. 3 *a* the intensity of the tones in the far ear are displayed relative to the near ear As can be seen from this figure the head shadow reduces the intensity in the far ear mainly f r 2000 and 4000 Hz. By inserting an ear plug in the far ear the head shadow effect increases considerably (Fig. 3 *b*) Using the VR udi met r this effect can be utilized for detecting monaural deafness in small children

The artificial head was also used to elucidate the change in tone intensity at near and far ear induced by the normal movement of a child's head during test session. A movement of the artificial head up to 5 cm in all direction including 20 rotation from its original place was used to simulate the movement of the child's head The recordings showed as an average a ari tion of 3 dB in sound pressure level with 10 dB as maximum variation t 4000 Hz.

Primarily our apparatus was designed for measuring the monaural sound field threshold for pure tones. However by switching the tone stimulus and the picture from side t side it could lso be used for determining the binaural tone threshold with the conditioned orientation eye reflex accord ing to Suzuki & Ogiba. By som other simple arrangements the equipment

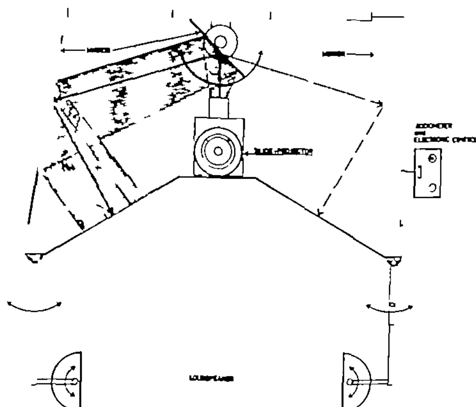


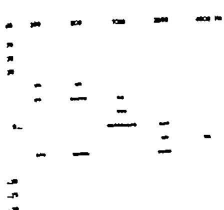
FIG. 2 Block diagram of the VR-audiometer

10 normal subjects whose threshold hearing level in both ears was in the range of ±5 dB re ISO standard threshold of hearing for pure tones with headphones. The loudspeakers were placed 15 cm lateral of the subject's ears, i.e. at 90 degrees azimuth. The ear not under test was blocked with an insert ear plug and covered with an external muff. The thresholds were determined by the method of limits. The mean values of the attenuator settings for both ears were averaged. The sound pressure levels corresponding to the attenuator settings for the minimum audible field were read when the subject was not present. These values served as reference level for the hearing tests on children (Table 1). The actual testing of the children was performed in a sound proof room. The calibration was finally rechecked on the same subjects who served in the anechoic room.

The placement of the loudspeaker lateral of the child's head makes it possible to utilize the head shadow effect in separating right and left ears tone thresholds in spite of the absence of masking. In normal subjects we can expect rather identical sound field curves for both ears and thus any

TABLE 1 *Mean monaural minimum audible sound field in sound pressure re 0.0002 dyne/cm² for 10 normal adults with the VR-audiometer*

250 Hz	500 Hz	1000 Hz	2000 Hz	4000 Hz
19	7	1	−3	−3.5



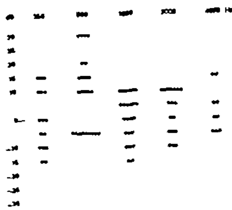


Fig. 4. Fig. 5.

Fig. 4. Test-retest variation i threshold sensitivity. Eight hard-of-hearing subjects (16 ears) between 1 and 3 years f ge. Each dot represents the diff rence between first and second test i dB for each subject.

Fig. 5. Comparison between t e thresholds bt ined with VRA nd earphone diometry. F fteen hard-of-hearing ubjects (26 ears) at 1000 Hz bet een 8 months and 2 / years of ge. Each dot represents the difference between first and second test in dB for each subject.

Our six age groups consisting of 120 otologically normal children could all successfully be tested in one session using visual reinforcement audiometry.

However the number f normal hearing children in these groups was rather small. Therefore we included an analysis of 935 investigations on children with suspected r verified hearing loss. The VRA technique was f und to be very useful in that all children but 10 of the 935 could successfully be tested.

The reliability of the threshold measurements judged by test retest results of VRA on eight hard-of hearing children (16 ears) in the age group of 1 1 3 years old was good as can be seen in Fig. 4. The retest was made about one month later. Another way of checking the reliability of VRA is to compare the threshold results obtained with VRA and ear phone audiometry. Such a comparison was made on 15 hard-of-hearing children (26 ears at 1000 Hz) in the age group 8 months to 2 /₂ years old. The two measurement were made in the same test session. The differences in thresholds are plotted in Fig. 5.

2. How does the hearing lev l in normal pre-school children compare with normal hearing f r adults when using VRA

The mean threshold curves for the different age groups measured with VRA are shown in Fig. 6. As can be seen the children s threshold fall short of normal hearing adults. This does not mean that the auditory organ in children is functioning worse than in adults but that children need some

could also be used for eliciting the acoustopalpebral reflex, for the arousal test for hearing aid evaluation in young children and as a peep show test (Dix & Hallpike 1947)

Procedure

As a rule the hearing session started with (I) visual reinforcement audiometry and finished with (II) conditioned orientation reflex audiometry

I Visual reinforcement audiometry

The child was trained to respond to a tone coming from the same side as the subsequent picture No attention was paid to the tone localization and concomittant eye movement The child was never instructed to respond in a particular way and neither was he expected to give a specific type of response Consequently a broad variety of responses could be observed These are discussed below under results

Children under 3 years of age were seated in the lap of the mother in front of the apparatus and were permitted to play with toys The testing procedure was not explained

Children over 3 years of age as a rule were not given any toys to play with but were told that they were going to hear a sound in one of the speakers, and after that see a picture.

The session started with a 500 Hz tone presented to the right loudspeaker at 30–40 dB above estimated threshold If the child below 3 years old responded he generally stopped playing for a moment and looked at the loudspeaker or the audiologist who pointed at the right window and projected a slide there The play with toys started over again and a new set of tones and pitcures were delivered on the same side a couple of times. By gradually reducing the intensity and watching the response of the child it was possible to obtain a tone threshold for the right ear The same procedure was then repeated for the left ear with the exception that the left speaker and the left window were utilized for this measurement

II Conditioned orientation reflex audiometry

The child was trained to the same combination of tone and picture but with the change that the sides of presentation of stimuli were randomly alternated The criterion for a correct response to the tone stimulus was now the immediate look at the picture or the loudspeaker on the same side as the presented tone Thus this method corresponds closely to that used by Suzuki & Ogiba.

RESULTS

1 Is visual reinforcement audiometry a successful technique for eliciting responses to auditory stimulations in normal and hard of hearing pre-school children?

(c) Some children learned very quickly that every tone was followed by a picture. In this group no expectation on the face of the child could be noted. No other evidence of reflex response than the immediate look towards the window could be found. We have called this reaction an orientation response.

(d) We have designated the fourth type as spontanous response. This type of response is most highly developed. This means that the child reports directly that he heard the tone and that he immediately understands what is going on. The spontaneous response could be expressed in many different ways from pointing or looking at the window to raising the finger imitating the tone, just smiling, nodding or saying "now".

4. How does the efficiency of testing the hearing of pre school children using visual reinforcement audiometry compare with that of conditioned orientation reflex audiometry?

For the otologically normal children the binaural tone threshold as judged by conditioned orientation reflex audiometry are shown in Fig 7. The results look very similar to those of VRA (Fig 6). However a detailed comparison between the threshold curves for the age group 1 and 4 years old determined with both methods demonstrated certain differences. In the youngest group both methods seemed to give similar thresholds. The group 4 years old on the other hand did considerably better with the VRA technique than with COR audiometry.

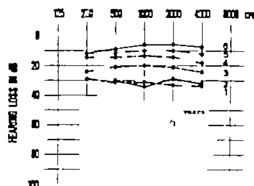
5. For how long a time during the test session could the interest of the child be maintained using these methods.

The 15 minutes time reserved for every child was long enough for determining the tone threshold with the use of both techniques. The visual reinforcement audiometry involved five correct responses to a minimum audible sound field on each side. The binaural threshold assessed with the conditioned orientation reflex audiometry needed five correct responses to a minimum sound field.

The reinforcements with the different slides kept the child's motivation in the test situation high. However it was easy to see that the children between 1 and 2 years of age did best during the first 5 minutes, and the older ones during the first 10 minutes.

DISCUSSION

Any response of the child to a sound stimulus was accepted with the visual reinforcement audiometry. This made it easier to get a threshold response. On the other hand, the inherent difficulties of the method were also evident. The evaluation of the responses in terms of true responses depended very much on the qualifications of the audiologist. Erroneous conclusion based on unconscious changes of facial expression of the audiologist could influence the child to respond to these gestures and not to the sound.



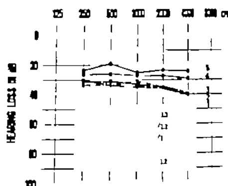


Fig 6 Fig 7

Fig. 6 Mean monaural sound field threshold curves for diff rent age groups obt ined with VRA.

Fig. 7 Conditioned orientation reflex audiometry Mean binaural threshold curves for different age groups.

what louder sounds before they respond With other words the mean threshold curves reveal hearing nearer that of adults as the children grow older indicating maturation of response.

3 How do the responses vary in different children of different age groups when using VRA?

The aim of the visual reinforcement was of course, to make the rather meaningless pure tones more interesting to the children The response of the children in the two youngest age groups was very much dependent on their ability to associate the tone stimulus with the picture. The fact that the child was not expected to give a specific type of response was adventageous, and he became very cooperative and active even though the audiologist was a stranger to him and it was the very first time he had his hearing tested. Because of the limited time we expected the child to maintain the interest and motivation for the test only a few pairings of auditory stimulus and visual reinforcement were presented for training purpose Consequently the children gave a variety of different responses. The four main types of responses encountered are listed below

(*a*) The most primitive type of response could be classified as *reflexive behavior* A first sign that the tone had been heard was that the intense and often somewhat expectant expression on the child's face changed A variety of movements of the shoulders or head were accepted as well as facial expressions such as wrinkling of the forehead, widening of the eyes, jerks in the lips or change of rate of eye blinking The reflexive type of response was mainly found in the first age group (below 1 year)

(*b*) The youngest children did not immediately understand the connection between the tone and the picture but they gradually learned The first step to comprehension was a look towards the loudspeaker next they associated the tone wih the audiologist and faced her These types of "What is it" reflex we have called investigatory responses.

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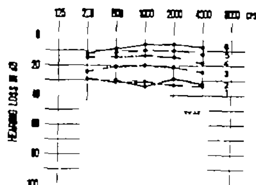


Fig 6

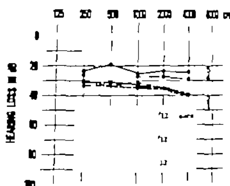


Fig 7

Fig 6 *Mean monaural sound field threshold curves for different age groups obt ined with VRA*

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positi r Befunde haben wir anstatt uns des Schallokalisationsvermögens oder bastigen Seitenblickreflexes zu bedienen, eine nicht richtungsbedingte Technik a gewandet bei der wir ler verschiedene Typen spontaner durch einen Prüfton erzeugten, Reaktionen akzeptieren.

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DISCUSSION

A Laskiewi z The peep-show was designed in 1947 by Mrs M. R. Dix to over come th great practical difficulties of obtaining *rapidly* a reliable pure tone udiogram in y ung children. Thi method of testing hearing in children is still on in whi h there are few f cilities *for repeated* bservation by a number of audi logists Trenquer St tten, Wishart (Toront) Mitri owicz (Warsaw) Paltas, Lang Orban, and Czanyi (Hungary) ha e stated that Mrs Dix s idea in peep-show testing of hearing in children is f great practical value having a particular place in the armory of udiologist.

J E Bordl p One f the most important points mphasized by M Lidén i that he i not act lly obt i ing thresh lds for sound stimuli i the young child. It takes considerable i tensity of stimulus in such hildren t btain their attention, and such a inten ity requirement dimi ishes as the child grows. Such

It was also easy to overlook a child's way of responding if one expected the child to respond in a certain way.

The thresholds obtained for the older children by the COR-technique seemed to be influenced in a favorable direction by practice. In the younger group no such factor was noticed. It was quite evident that the COR technique with the tones alternately presented in the right and left speaker gave poorer thresholds than with the more simple VR method. For some children the COR technique was too difficult and as a result some stopped giving responses at all. The children became tired quickly and needed retraining or reinstruction with the COR technique more than with VRA.

VRA should not be confused with the slide show audiometric technique described by Lloyd (1965) nor with the peep show by Dix & Hallpike (1947). Although there are some similarities in the equipment the fundamental difference is found in the way the child is responding to the stimulus. In VRA the child is not instructed to respond in a particular way. Thus no active co-operation of the child is required as in the other methods.

CONCLUSION

Visual reinforcement audiometry has proven to be a successful technique for eliciting responses to auditory stimulation. Hard-of hearing children could reliably be tested from about 6–8 months old. Compared to adults the youngest children needed somewhat louder sounds before they responded. With other words the mean threshold curves revealed hearing nearer that of adults as the children grew older indicating maturation of response.

Conditioned orientation reflex audiometry also appeared to be a useful technique for measuring hearing of pre school children. This test however was more difficult than VRA. The sound localization task proved too complicated for many children and louder sounds were often required for a correct response. This was particularly evident in the older age groups in which COR audiometry gave poorer thresholds than VRA.

RÉSUMÉ

L'audiométrie tonale avec renforcement visuel est une modification et une simplification du réflexe conditionné d'orientation. Au lieu d'employer la capacité de localisation du son ou le réflexe de déviation de l'œil comme critère de réponse nous employons une technique non directionnelle et acceptons quatre différents types de réponses spontanées.

ZUSAMMENFASSUNG

Die Schaukastenaudiometrie (Visual reinforcement audiometry) *ist eine neue* Methode welche für die Prüfung des Gehörs sehr kleiner Kinder gut geeignet ist. Diese Methode ist eine Verbesserung und Vereinfachung der von Suzuki und Ogiba ausgearbeiteten *conditioned orientation reflex audiometry*". Zur Erreichung

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THE NYSTAGMUS THRESHOLD BASIS OF VESTIBULOMETRY

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New experimental research is presented on the determination of the parameters of stimulation and of measurement of the vestibular responses recorded by ENG with special mention of a procedure allowing the checking of the accuracy of the stimulating devices.

Because of its extreme complexity vestibular diagnosis requires very simple and accurate methods of investigation. Tests have to be always identical, reproducible and measurable. They must permit also clear recognition of the limits between the normal and the pathological. A good example of clinical investigation of a sensory apparatus is tonal audiometry as it is practised nowadays, which can be used not only for qualitative, but also quantitative appraisals of its function.

The purpose of the Liminal Rotatory Test (LRT) we have earlier described (Montandon *et al.* 1954, 1956, 1961, 1965) is to define by means of a constant angular acceleration of 1°/s², 3°/s² and 6°/s² from 0 to 90°/s of rotation speed, with an ENG record, at what level the nystagmic signal emerges. From that point it becomes possible to quantify the vestibular function in the sense of a vestibulometry comparable with audiometry. Therefore it is essential that the nystagmic threshold, which appears in form of an objective reaction, should be as exactly defined as the audiometric one which is a subjective sensation and which was defined by a statistical analysis.

Definition

Despite the diversity of the criteria used by the authors—for example the sensation of rotation, the postrotatory nystagmus with extrapolation of a theoretical threshold, or the perrotatory nystagmus of angular acceleration—the value of the vestibular threshold has nevertheless been established around 1°/s²: Mulder (1908) 2°/s², Buys (1910) about 1°/s², van Wulfften Palthe (1922) 2°/s², Arslan (1934) 0.5–1°/s², Groen & Jongkees (1948) 0.5°/s², Hulk & Jongkees (1948) 2.5°/s (0.75 to 5°/s), Hilding (1953) 0.7–1°/s² (0.25–3°/s²), Hood (1966) 1–2°/s², Kaphan (1968) 1°/s². Others, like de Vries & Schierbeck (1953) and Roggeveen & Nijhoff (1956) have tried to obtain an instantaneous threshold using very short stimulation of

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apparent improvement in hearing in the growing child has lead many observers to think a growing child has improving hearing. This sometimes leads to false conclusions. Our experience is that with surmounted evoked cortical potentials following sound stimuli, values nearer the true thresholds in children under 2 / years are obtained, than by the use of various type tests.

Finally I would like to ask Dr Lidén at what intensity values he would accept an audiogram as normal in the case of a 6-year-old child.

A Burian Wir haben eine Gruppe von 50 Kindern im Alter zwischen 6–12 Monaten mit der Methode der Baby Bett Audiometrie (nach Bisalski) untersucht. Dabei ergab sich eine Hörschwelle zwischen 30–40 dB Wir haben die gleiche Gruppe von Kindern mit Hilfe der Evoked Response-Audiometry einer Kontrolluntersuchung unterzogen Dabei ergab sich eine objektive Hörschwelle von 20–25 dB Diese objektive Hörschwelle fällt mit zunehmendem Alter ab und erreicht in Alter zwischen 4–6 Jahren eine Schwelle von 0–5 dB

G Lidén (Reply) to Mr *Laskiewic* The reason that I did not discuss the paper of Dix & Hallpike is that I did not want visual reinforcement audiometry to be confused with their method. In visual reinforcement audiometry the child is not instructed to respond in a particular way Thus no active cooperation of the child is required as in the peep-show technique according to Dix & Hallpike

To Mr *Bordley* At 6 years of age the tone thresholds are very close to that of adults.

TABLE 1 *Vestibular nystagmus threshold of angular acceleratory nystagmus recorded on 139 normal human subjects*

Author	Number of cases	Mean value (calculated)	95 % of the cases are comprised between the two values
Flowbach, 1955	34	0.77 °/s	0.59–0.91 °/s
Lumeaux, 1958	105	0.92 °/s	0.76–1.06 °/s

Experimentally that couple may be demonstrated using the stereotaxic method on rabbits. By means of a continuous electric stimulation of the Diencephalic Nystagmogenic Area, at a frequency of 35 Hz, a nystagmic reaction is induced at a definite level of intensity (0.5–1 V). This diphasic nystagmus of central origin is absolutely similar and correlated to the nystagmus provoked by rotatory stimulation *threshold* latency time of a few seconds, increase of the frequency and maximum frequency rate corresponding to the intensity of stimulation, and poststimulatory nystagmus evoking the postrotatory nystagmus (Monnier M. & Montandon, P., 1962 Montandon, P 1964 Montandon P & Monnier M 1961 Montandon, A *et al* 1963, 1965, 1967)

The same correlation must be realized in clinical work using a continuous angular acceleration by means of a turning system In order to obtain a constant rotatory stimulation, reproducible and of a definite value allowing a clinical valuation of the nystagmic threshold, every turning device has to be carefully standardized and subsequently controlled

In this connection it must be recalled that the inscriptions on the dial are usually not accurate that the setting of the equipment is frequently out of order and that the tachometer connected directly on the motor does not give reliable information on what happens to the turntable itself The only measurements which can be considered reliable are those made directly on the turntable

During a two-year period of trial in our laboratory many testing procedures of the *accuracy of the rotating devices* have been checked, showing the considerable importance of this factor in clinical vestibulometry Practically we suggest a simplified chronometric method which may be used by every operator (Table 2 and comments.)

TABLE 2

Angular acceleration (1 °/s)	Time in seconds for			
	½ turn	1 turn	1 ½ turn	2 turns
Calculated time	19.0	26.8	32.9	37.9
Lower limit	18.4	26.1	32.0	36.9
Upper limit	19.6	27.6	33.8	39.0

0 4 s That procedure does not consider "the time element which is an important factor with a definite latent period of a few seconds at the low rates (Hilding 1953)

Our personal definition of the nystagmic threshold as it appears in the limits of the Liminal Rotatory Test must be completed as follows in order to avoid any confusion *The threshold of the vestibular nystagmus is a frequency threshold* It corresponds to *the lowest grade of intensity of a continuous and constant stimulation which is able to provoke and to maintain until the end of the stimulating period a nystagmic reaction of a definite direction and of one beat per second (1 H)*

Remarks

I This definition can also be applied to the central nystagmus issued from an electrical stereotaxic stimulation of the Diencephalic Nystagmogenic Area (DNA) as well as to the nystagmus provoked by an angular acceleration The values in /s² should be replaced by measurements in volts (0 75–1 v)

II The nystagmic reaction is a *rhythmic process* (Monnier 1967) of di phasic beats, with a slow and a rapid phase which occurs in three stages latency time, start of response and period of state (maximum frequency) *Only the last period of a steady maximum frequency must be taken into consideration to determine the threshold* The two preceding periods, possibly reaching 10 to 20 seconds, are not suitable for a quantification. *A minimum duration of 20 to 30 seconds of stimulation at a constant intensity is consequently required*

III Every reaction exceeding the period of stimulation corresponds to a supraliminal intensity level

IV *Other parameters, like amplitude or speed of the slow phase* are not considered (frequency threshold)

According to the statistical analysis of two different series of researches carried out in our laboratory the threshold of the angular acceleratory nystagmus recorded by ENG in complete darkness has been established at the values referred in Table 1

It must be emphasized that

1 The level of 1 /s² chosen as a *clinical threshold* is situated at the upper limit of the real threshold, which is usually lower

2 Between the normal and the lowest pathological value at 2 /s² the minimum gap reaches at least 100% which represents a very significant margin of security

Parameters of Stimulation

The existence of an accurate correlation, a so-called stimulationresponse couple is a necessary condition to define the threshold of the nystagmus.

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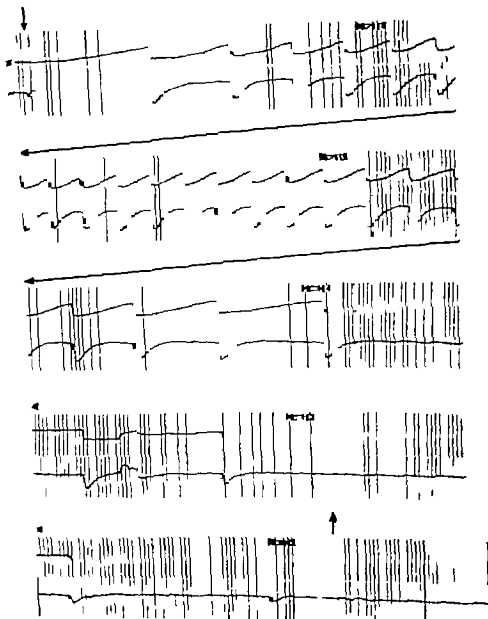


FIG. 2. Electric stereotaxic stimulation of the right DNA just below threshold (0.4 Hz) Direct coupling amplification (DC) ~ alternating coupling amplification (AC)

↓ Start of stimulation. ↑ End of stimulation.

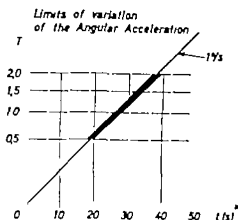


FIG. 1 Limits of allowed variation of the angular acceleration.

Simplified Chronometric Method to Check the Rotary Stimulating Device (for 1 /s²)

1 Check constant speed (90 /s) and adjust if necessary Measured time for 10 turns must be 40 seconds Previously use acceleration of 1 /s approximately

2 Check total acceleration time and adjust acceleration value (1 /s²) if necessary The time must be 90 seconds. (Points 1 and 2 not necessary if device functions correctly)

3 Measure the time the turntable takes to make the first halfturn Stop the device only after it has reached the speed of 90 /s.

4 Repeat point 3 for the first turn turn and a half double turn

5 Repeat the whole test for different loads of the rotating chair

The obtained values are compared with Table 2. *The four points have to fall within the given limits* If it is not the case the stimulating device cannot be considered as adequate to perform the Liminal Rotatory Test or to do *any* research on the response threshold

Remarks

The allowable errors in chronometry have been taken into account in the given limits

Before starting the test two marks have to be stuck on the turntable exactly 180 from each other

This test takes into account the constance, the exact value (1 /s²) and the reproductibility of the stimulation

The lower and upper limits given are worst-case values (errors all adding up in the same direction They correspond to an error of about 5% of the acceleration value (1 /s²) and 0 1 s timing error The measured time should be closer to the calculated theoretical time as is the case for the practical measurements we performed on the Girograph in our laboratory (Fig 1)

Parameters of Observation

At the present time the nystagmus is most commonly recorded by the corneo-retinal potentials derived from periocular skin-electrodes (ENG) Our recording system includes two modes of amplification the direct coupling (DC) which allows recording of the slow phase as well as the rapid phase, and the alternative coupling (AC) with a time constant of 0.3 s, which does not reproduce truly the eye movement but gives a perfectly stable ENG trace. Instead of DC, it should be sufficient to use only one AC amplifier with a longer time constant (e.g 1.5 s)

The main parameter of the nystagmic threshold is the frequency the nystagmus being a rhythmic process, therefore the defined threshold is a *frequency-threshold* (Figs. 2 and 3)

As for the amplitude of the beats, variations may occur depending on the procedure of recording, the type of apparatus, the filtration, and the placement of the electrodes. Therefore, this criterium is not valid.

The slow phase is often hardly recognizable if not accompanied by the fast phase of the nystagmus. According to our experience, the speed of the slow phase appears to be graphically correlated with the frequency if the amplitude remains constant. Its value is consequently submitted to the same uncertainty as amplitude It is not a suitable criterium for the threshold.

As far as the choice of a method of recording is concerned it must be pointed out that every procedure using *visible light* even red light, *considerably disturbs the amplitude and the frequency of the nystagmus* Therefore the recording has to be carried out in complete darkness.

Clinical Applications

What is the practical meaning of the nystagmic threshold? It was experimentally demonstrated that each one of the DNA and of the peripheral labyrinths is normally responsible for one direction of the nystagmus, to the right or to the left. Each DNA is strictly unidirectional while each labyrinth is only partly unidirectional o at unilateral preponderance nearby the threshold. In order to differentiate the reaction of each labyrinth separately it is necessary to use very weak, precise, and constant acceletory stimulations f long duration at the threshold value (1 $/s^2$) From the nystagmic threshold it is then possible to evaluate the loss of function, the grade of central compensation, the masking intensity of a latent or spontaneous nystagmus, and to follow by an exact quantification the evolution of any vestibular dysfunction from central as well as from peripheral origin. Two years ago we published an ENG semeiology based on 12,000 traces representing 2000 patients tested by this method of vestibulometry (Montandon, 1966)

Rabbit 1017 ES DNA right, 0.75 V



Fig. 3 Elect l t reot xic timul ti n of th right DNA bo thresh ld (27 H) plus poststimulat ry nystagmus. — Direct coupli g mplific ti (DC) ~ alternati e coupling mplification (AC)

↓ Start of stimulation. ↑ End f stimulation.

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DISCUSSION

S. Khilnashvili: M. Montandon has recorded central nystagmus induced by direct stimulation of the CNS and studied changes of this nystagmus under the influence of rotatory accelerations. Such studies may help to understand the mechanism involved in central nystagmus in man. But I am not sure that threshold studies will be a valuable in cases when the vestibular end-organ is impaired. In such cases the study of the slow component of the nystagmus under conditions of supraluminal stimulation seems to be more important.

J. J. Groen: Why does M. Montandon insist on the angular acceleration as the stimulus for determining the vestibular threshold? The acceleration takes long time to produce its maximum effect. Actually the product of acceleration and the duration of its application to provoke the first nystagmus stroke (slow phase) is a constant, which is equal to the minimum angular velocity. This one constitutes the real vestibular threshold. So why not use an impulse as in cupulometry which produces the wanted value immediately?

G. F. Groen: La recherche pour préciser le seuil de la réponse nystagmique est de la plus grande importance et pour la réaliser il est évident que le stimulus doit être régulier et rigoureusement mesurable. C'est pourquoi nous utilisons la stimulation sinusoïdale au cadre pendulaire. Ce moyen plus facile à réaliser permet de déterminer le seuil et donne ainsi en clinique un renseignement très important.

L. B. W. Jongkees: Mr Montandon defines the threshold for his experiments and examinations as the stimulus that gives a nystagmus till the end of the stimulation. This does not seem to me to be possible since during accelerating

RÉSUMÉ

Nouvelles recherches expérimentales sur la détermination des paramètres de stimulation et de mensuration des réponses vestibulaires enregistrées à l'ENG, avec une mention spéciale d un procédé permettant de vérifier le degré de précision de l appareillage utilisé

ZUSAMMENFASSUNG

Neue experimentelle Untersuchungen über die Bestimmung der Reizparameter und die Messung der registrierten vestibulären Antwort (ENG) mit spezieller Erwähnung einer Methode zur Kontrolle des Genauigkeitsgrades der verwendeten Einrichtungen

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Acta oto-laryngologica 67 363–369 (1969)

THREE DERIVATIONS IN ELECTRONYSTAGMOGRAPHY

M. Pansini and I. Padovan

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In order to be able to detect in the simplest possible manner and in part also to analyse the changed direction of nystagmus, three derivations for recording nystagmus have been introduced. Three bipolar leads are connected to three electrodes. The magnitude of the electric effect in one derivation is directly proportional to the corneoretinal potential in the second inversely proportional to the square of distance of the lead points from the rotating dipole both are corrected by calibration. The third magnitude of the electric effect is in direct proportion to the cosine of the deflection angle which exists between the rotating dipole and the line of derivation. It is thus possible to mark in an isoceles for each derivation the amplitude of the deflection of the eye from the central point.

Electronystagmography has not, till now had a generally-accepted standardisation, only conventions. The active electrodes are situated at each outer canthus of the eye and the ground electrode is placed on the forehead in order to obtain the horizontal derivations for both eyes at the same time. In horizontal nystagmus the right-beating nystagmus is positive and lies above the zero line, while the left beating nystagmus is below the zero line.

The calibration is also conventional, having the patient *alternate his gaze* between two points representing a 10° arc of swing of the eyes so that *the* amplitude in the nystagmogram is 10 mm.

Three-channelled vectographic electronystagmography

In order to see and analyse also the other *derivations of the* nystagmus besides the horizontal one we introduced three directions in the registration of the nystagmus. By doing so, we did not neglect the general desire for standardisation in nystagmography. We follow this form strictly so that we do not change either the number or the position of the electrodes or the raw or conventional channels.

The technique is as follows. The ground electrode situated on the forehead also becomes active, so that we have at the same time three channels instead of one, forming an isocele triangle. *In this way* every direction of the nystagmus can be situated and *from* the vectogram its direction and intensity determined.

From the three electrodes have three bipolar derivations

First channel electrodes 1–3
Second channel electrodes 1–2
Third channel electrodes 3–2

stimulation of constant magnitude the nystagmus always disappears after some 40 seconds.

A Montandon (Reply) We are grateful to our colleagues for their interesting discussions which show what important confusion still remains concerning vestibular threshold and especially the threshold of the nystagmus. Concerning our definition we must insist on two basic notions the nystagmus is a rhythmic process and the threshold has been defined by the frequency of the ocular beats recorded in complete darkness by ENG. Experimentally the central permanent stereotaxic stimulation of the DNA gives rise to *a quite similar reaction* as the permanent application of a constant angular acceleration that is unidirectional nystagmus, with slow phase and quick phase frequency threshold corresponding to our preceding definition and progressive increase of intensity at supraliminar intensity levels of stimulation Now we may add separately

To Mr *Khechinashvilli* *We did not find any more information* at constant amplitude of the nystagmus by considering the speed of the slow phase than only the frequency (as demonstrated by slides)

To Mr *Groen* The Mulder's law of the product of time x intensity of stimulation is only valid during the setting up of the nystagmic reaction but not at the maximum frequency level.

To Mr *Greiner* I agree completely with the angular acceleratory threshold he has demonstrated in the pendular test.

To Mr *Jongkees* We cannot compare the postrotatory (or poststimulatory) nystagmus used in cupulometry with the *perrotatory nystagmus* We are recording *during* the *permanent stimulation* whether central or peripheral, by angular acceleration in the Liminal Rotatory Test the duration of the perrotatory nystagmus is normally of at least 80–90 seconds if you accelerate at I $/s^2$ to constant speed of 90 degrees per second of 180 seconds if you accelerate till a speed of 180 degrees per second and of many minutes or even longer if using a central stimulation There is practically no exhaustion of a nystagmus obtained by permanent stimulation contrary to the nystagmus succeeding to an instantaneous and strong shock (after discharge)

The quantity of the electric response in each channel is

1 Directly proportional to the corneoretinal potential

2. Indirectly proportional to the square of the distance between the issue points of the rotating dipole.

3 Directly proportional to the cosinus of the angle of derivation which is formed between the direction of the rotating dipole and the line of deriva tion and line of the channel.

Calibration

The first channel gives the sum of the deviation of both eyes and is calibrated in the conventional way having the patient alternate his gaze between two points representing a 10 arc of swing of the eyes which is determined as the amplitude of 10 mm To simplify the method, we did not calibrate separately the second and third channels but reduced during the horizontal calibration the amplitude in the second and third channels to 5 mm

Because of the reduction of the electrical effect by the square of the distance, the second channel registrates prevalently the deviation of the left eye and the third channel deviations of the right eye.

Analysis of the three-channelled electronystagmogram

1 In horizontal deviations of the eye the amplitude in the second and third channels is equally large but of the opposite direction and their annulation proves that the eye moves only in the horizontal direction.

The horizontal nystagmus is seen in Fig 5

2 In oblique nystagmus the amplitude in the second and third channels will not be equally large. The more the direction of the eye movement becomes vertical to the direction of the channel the smaller will be the amplitude in this channel

In this way is registered an alternative fixation of two points under an angle of 10 but in an oblique line.

Fig. 2. Three lectrodes represent three channels. Their connections and their positi direction are indicated.

Fig. 3. Horizontal deviation of the eye to the right and its projections t the three channels.

Fig. 4. Calibration. The e fixes alternati ly two points on horizontal line

Fig. 5 The left beating nystagmus. Between the t thick lines is the time f 1 second.

Fig. 6. The obliqu movement of the eye and the projection in three channels.

Fig. Alternati fixation of two points. The eyes mov from right and below to the left nd upwards little above the horizontal line.

Fig. 8. Left-beating nystagmus. It is not pure horizontal ystagmus. According t the direction it corresponds t the movement of the eyes indicated in Fig 7

Fig. 1 Conv ntl nal horizont l hann l The positi pol i lt ted t the right uter canthu f th y the neg tl e t th left uter c thus, nd the grou d lectrode n th foreh d.

In a case where the direction of the deviation of the eye is perpendicular to the direction of a channel the pen movement in this channel is zero

In the conventional electronystagmogram with one raw channel the analysis of the amplitude of the nystagmus and the velocity of the fast and slow components is exact only if the nystagmus is completely horizontal. The more oblique the nystagmus the bigger the error there does not exist a fixed system for finding the error and of correcting it.

In the three-channelled electronystagmogram it is possible to include a simple computor which is able to give, on the basis of the findings in the three channels, the exact direction of the nystagmus, the amplitude of the nystagmic movements, and the velocity values of the fast and slow components of every nystagmic movement.

3 In pure vertical nystagmus there is no response in the first channel while the second and third channels show the same amplitude and direction.

If the nystagmus is vertical but with a slight inclination to the right or left, there is a response also in the first channel which can, in the case of only raw derivation, lead to a false interpretation.

CONCLUSION

Electronystagmography with three channel does not change *the conditions* of registration in the conventional raw derivation.

The ground electrode on the forehead is transformed to the active electrode so that three channels forming an isocele triangle are obtained.

It is possible in this way to follow very direction of the *nystagmus and* to determine from the vectogram the exact amplitude and *velocity of the* slow component also in the case when the nystagmus is not horizontal

By this method of registration of the nystagmus, errors in measuring the intensity of the nystagmus when it is not horizontal and the *error of inter* preting the v rtical nystagmus as the horizontal can be *avoided.*

Three-channelled electronystagmography is applied in the registration of spontaneous nystagmus, of all sorts of *positional* nystagmus, and of induced nystagmus. It has become a routine electronystagmography

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Fig. 11 Left-beating stagmus but with the directio perpendicular t the second hannel.

Fig. 12 Left beati g ystagmus but *with the directio* perpendicula to the *third* cha nel

Fig. 13. The movement of the eye i the ertical direction d the projection *in the* channels.

Fig. 14 The mov ment f the ey between the two points on ertical line.

Fig. 15 Vertical stagmus which would not be registered in con entional raw derivation; in the first channel there is no pen movement.

Fig. 16 The ertical *nystagmus* upwards but with a slight incl nation t the right There ppears lso response in the first cha nel. I the second hannel *the* ampl tude is bigger than in the third one because it correspond to the ectogram

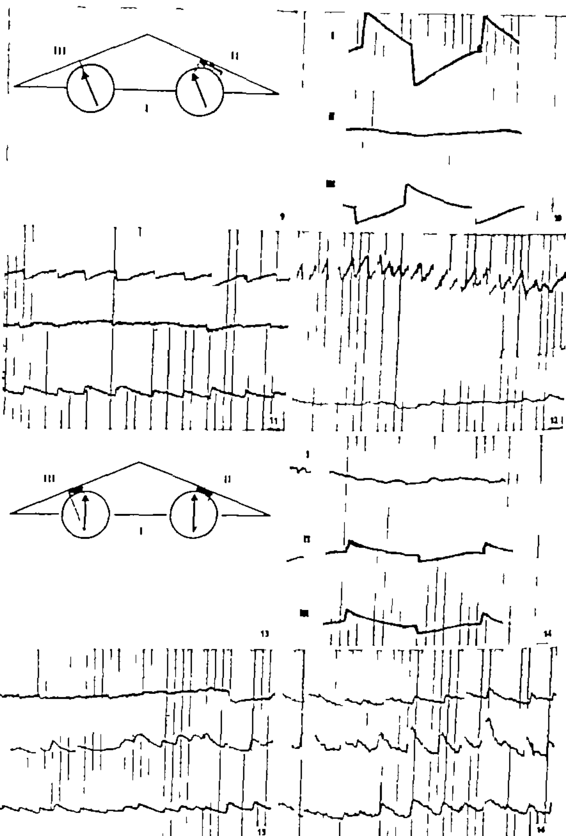


FIG. 9 The m m nt f the eye in the directi perpendicula t the third h nn l and th projecti n in th cha nels.

FIG. 10 Altern ti e fixation of th tw points n ll perpe dicular t th second chann l.

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Three-channelled electronystagmography is applied in the registration of pontaneous nystagmus, of all sorts of positional nystagmus, and of induced nystagmus. It has become a routine electronystagmography

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Fig. 14 The mov ment f the eye between the t point on vertical line

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In the dissociated nystagmus in which, instead of beating harmony of both eyes, only one eye will move while the other remains motionless, moves slightly or in the opposite direction three-channelled electronystagmography will warn us that we have to deal with a dissociated nystagmus because it will not be possible to compose the vectogram In such a case registration by the other differentiated channels will be applied

RÉSUMÉ

En vue de découvrir d une manière simple et de pouvoir analyser partiellement la direction du nystagmus changée les auteurs ont introduit trois dérivations dans l enregistrement du nystagmus. Au moyen de trois électrodes, les auteurs ont fait trois dérivations bipolaires. La dimension de l effet électrique dans une dérivation est directement proportionnelle au potential cornéo-rétinal la seconde est inversement proportionnelle au carré de la distance des points de conduite du dipol rotatif mais les auteurs les corrigent par la calibration La troisième dimension de l effet électrique est directement proportionnelle au cosine de l angle de la déclinaison existant entre la direction du dipol rotatif et la ligne de dérivation De cette façon il est possible de tracer dans un triangle isocèle pour chaque dérivation l amplitude de la déclinaison positive ou négative de l'oeil du point central.

ZUSAMMENFASSUNG

Um die Richtungsveränderung des Nystagmus möglichst einfach zu beobachten und teilweise auch zu analysieren werden drei Kanäle in die Registration des Nystagmus eingeführt Von 3 Elektroden werden die 3 bipolaren Derivationen ent nommen Die Grösse des elektrischen Effekts in einem Kanal ist direkt proportionell dem korneo-retinalen Potential in einem zweiten indirekt proportionell dem Quadrat der Entfernung der Derivationspunkte vom rotierenden Dipol, was durch Kalibrierung ausgeglichen wird. Im dritten ist die Grösse des elektrischen Effekts direkt proportionell dem Cosinus des Deklinationswinkels der zwischen der Richtung des rotierenden Dipols und der Derivationslinie besteht So ist es möglich, dass man für jeden Kanal in einem gleichschenkeligen Dreieck die Amplitude sowohl der positiven als auch der negativen Abweichung des Auges von dem Mittelpunkt eintragen kann.

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DISCUSSION

G F Greiner J pense qu la technique de Mr P dovan permet d mieux in scrire le ystagmus girat ire et de fi er les différents composants du movement du globe oculaire ce qui en fait n est pas réalisé par l'ENG habituell. J'aimerais savoir quelles sont ses électrodes et leur emplacement permettant les dérivation bipolaires.

I Padovan (Réponse) à Mr *Greiner* En ce qui co cerne les électrodes elles sont posées de la manière usuelle c'est à dire bitemporale et sur le fro t. Le problème principal étant le troisième canal. Nous faisons nos recherches en collaboratio avec l'Institut de télécommunication.

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OBSERVATIONS UPON THE NERVOUS MECHANISM OF VESTIBULAR HABITUATION

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Rotational tests carried out upon a number of ballet dancers have shown their nystagmic threshold responses to angular acceleration to be normal when determined in darkness, but considerably raised in the presence of optic fixation These results indicate that optic fixation plays an important role in vestibular habituation in man and this, in turn calls for some reassessment of current views on its nervous mechanism.

Although, in contrast to caloric tests, rotational tests have limited localising value inasmuch as it is impossible by their means to examine separately the function of each labyrinth they do possess one singular advantage in that they enable precise and accurately graded stimuli to be applied at will. In this way the physical forces engendered within the canals may be determined and systematically related to the resultant nystagmic and other induced responses.

A feature of particular value in this respect is the ease with which rotational tests make possible the determination of the absolute sensitivity of the semi-circular canal mechanism The information thus provided supplements that of the caloric tests, and has proved to be of considerable value in studies of vertigo and in particular of vestibular habituation which is the subject of this communication

The cupula of the horizontal semi-circular canals, of course responds only to angular acceleratory stimuli This calls for a rotating chair of high precision That which we have used is servo-mechanically controlled and by means of it angular acceleration from 0 1 to 10 s^{-2} with intermediate values may be applied at will The cupular response to a sustained angular acceleration is a progressive increase in its angular deflection to a maximum value which is approached asymptotically and reached effectively after some 20 seconds. For this reason it has been our practice to apply angular accelerations for 20 seconds followed by prolonged periods of rotation at constant velocity The resultant nystagmus is recorded electro-nystagmographically in total darkness. The procedure for the determination of canal sensitivity is exemplified in Fig 1 This shows the nystagmic responses of a subject to four different angular accelerations each beginning, for convenience with the chair at rest It will be seen that the lowest acceleration is sublim

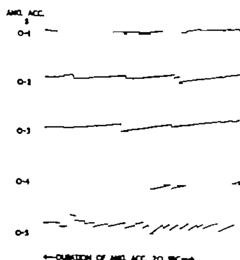


FIG 1 Nystagmic response of normal subject recorded with eyes ope I darkness t four angula acceleratory stimuli f 20 seconds duration.

inal and no nystagmus is detectable upon the recording At 0.2 s⁻ nystagmus begins to make its appearance and increases with further increase in angular acceleration.

The threshold response for this subject, theref re, lies somewhere between 0 1 and 0.2 s^{-2} and this figure provides a clear definitive measure of threshold sensitivity Th actual test procedures we adopt in practice is shown in Fig 2. From rest, the chair is accelerated at 0.5 s⁻ for 20 seconds, followed by a period of 60 seconds rotation at constant velocity In the normal subject this induces a clear supraliminal nystagmic response. The chair i then accelerated at 0 1 s^{-2} for 20 seconds again, followed by 60 seconds rotation at constant velocity and this, in turn, is followed by a deceleration of 0 1 s^{-2} and so on with progressively increasing or decreasing alues f angular acceleration or deceleration until a clear nystagmic response can be detected upon the recording In this way not only can the threshold response be bracketed in much the same way as one determines the threshold f hearing, but this procedure also enables any directional preponderance present to be expressed in terms of threshold sensitivity in one direction r another Normal threshold sensitivity appears to be of the orde of 0 15 s⁻ This attests to the remarkable sensitivity of the cupular mechanism and indeed it seems not unlikely that in some individuals even higher sensiti ities might well be revealed with further refinements in rotating chair design

As a variant of this test procedure angular ecceleratory thresholds may be determined in the same way with the subject fixating a light spot attached t and rotating with the chair Optic fixation, of course, exerts a strong inhibitory effect upon induced nystagmus and, as a result, the stimu-

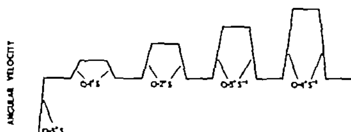


FIG 2. Test procedure used for the det rmination of nystagmic threshold response t angular acceleratory stimuli

lus magnitude has on the average to be increased ten fold in the normal subject that is to say to 1.5 s^{-2} in order to evoke a just detectable threshold response This factor of 10 we have referred to as the fixation index, since it would seem to provide a convenient expression of the magnitude of the restraint optic fixation exerts upon induced nystagmus, and it is with this that we shall be principally concerned in the foregoing Let us now turn to the main topic of this communication namely so-called vestibular habituation

As is well known repeated rotational stimulation about the vertical axis results in both man and animals in a progressive elimination of the reflex nystagmic eye movements which normally follow stimulation of the lateral semi-circular canals The generally accepted view of the phenomenon is that it may be identified with habituation which Thorpe (1950) has defined as "an activity of the central nervous system, whereby innate responses to certain relatively simple stimuli, especially those of potential value as warn ing of danger wane as the stimuli continue for long periods without un favourable results" In man the phenomenon finds its most common mani festation in ice skaters, ballet dancers and certain flying personnel who, in the course of their activities, are subjected to repeated rotational stimula tion As a result they appear to develop an immunity to the vertigo which would normally accompany excessive turning of the body in the inexperienced subject In the past a number of studies have been carried out upon ice skaters and ballet dancers, the most notable being those of Mowrer (1934) and of McCabe (1966) Both authors comment upon the absence of nystagmus and vertigo in their subjects, either as a result of rotational stimulation or irrigation of the ears with ice water

As a result of these and similar studies it has been widely assumed that the phenomenon has its origin in some central inhibitory mechanism which in some way not clearly understood, suppresses the normal physiological response of the vestibular end-organ.

Our own studies have been concerned with four ballet dancers, all of whom had had over seven years experience of professional dancing

Caloric tests were carried out on all four subjects according to the technique described by Fitzgerald & Hallpike and, in accordance with previous studies the responses were found to be either totally abolished or grossly reduced. This, as has been mentioned, has in the past been taken to imply bilateral abolition or reduction of canal sensitivity

The results of the rotational tests, however carried out in the manner described, are shown in the following table and tell an entirely different story

Rotational Tests
Average for normal subjects

Angular acceleratory threshold of nystagmus in in darkness	Angular acceleratory threshold of nystagmus with optic fixation	Fixation index
0.15 $^{-2}$	1.5 $^{-2}$	10

Ballet dancers

Subject			
1	0.1	8	80
2	0.2	5	25
3	0.2	10	50
4	0.2	8	40

Abo e are recapitulated the findings in normal subjects, namely angular acceleratory thresholds of nystagmus with and without optic fixation of 1.5 and 0 15 s respectively giving a fixation index of 10 Below are shown the results, from the ballet dancers. The nystagmic thresholds in darkness, although marginally higher than the normal average, nevertheless seem to fall within the normal range, indicating in contrast to the caloric test results unimpaired semi-circular canal sensitivity The thresholds obtained in the presence of optic fixation on the other hand are all considerably raised, giving fixation indices from 25 to 80

It would seem, therefore, that what has happened in these subjects is not a suppression of canal sensitivity but the development of an abnormal capacity for maintaining visual fixation designed presumably to stabilise the eyes in the presence of disturbing vestibular activity in other words a reinf rcement of optic fixation.

Similar results to these ha e recently been reported by Collins (1966) Collin investigations were carried out upon professional ice skaters. Amongst other studies he recorded the eye movements of the skaters, both with eyes open and closed, following pirouettes. He found that, as was to

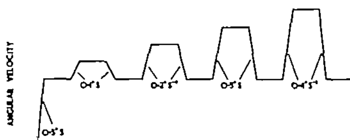


FIG. 2 Test procedure used for the determination of nystagmic threshold response to angular acceleratory stimuli.

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Our own studies have been concerned with four ballet dancers, all of whom had had over seven years experience of professional dancing

In any event our studies would appear to possess an immediate and practical application to clinical neuro-otology

The finding of absence of conventional caloric responses with normal rotational thresholds and high fixation indices is by no means peculiar to ballet dancers. We have recently described elsewhere its occurrence in 78 of 274 patients with a diagnosis of vestibular neuronitis (Dix & Hood) in addition it is a not uncommon finding in subjects with extensive flying or sea-going experience.

Coles & Knight (1961) for example, noted diminished responses to conventional caloric tests in a group of experienced naval divers they attributed this to long-term habituation to the vestibular stimulation that occurs in diving operations from small craft. Later Coles (1968) has noted that seamen patients in general frequently give minimal or no response to conventional caloric stimulation, although when repeated with the patient s eyes open in the dark under electro-nystagmographic recording there is often a response within normal range of magnitude (as judged by maximum speed of the slow component of the induced nystagmus) He interprets the habituation he has observed in seamen as being mediated by enhancement of the inhibition produced by visual fixation

Clearly therefore, bilateral abolition of the conventional caloric responses should never be taken to imply bilateral abolition or impairment of vestibular function. In all such cases the influence of optic fixation should be given special consideration. In this respect rotational tests carried out in the manner described can make a vital contribution to neuro-otological diagnosis.

RESUME

Des épreuves rotatoires vec des danseurs de ballet comme sujet nt montrées, que leur se il ystagmiqu à l accélératio angulaire est ormal, quand il est déterminé dans l obscurité mais qu il est considérablem nt élevé en présence d'un fi tion optique Ces résultats indiquent que la fixation opt que joue un rôle important dans l'habituation vestibulair et demandent pour ette raison une réexamin tion des vues courantes concernant l mécanism nerveux de l'habitua t on estibulaire.

ZUSAMMENFASSUNG

Drehprufung mit Balletttänzern zeigte ei en rmalen Schwellenwert für den Beschleunigungsny tagmus in Dunk lheit Im Gegensatz dazu ist der Schwellen wert mit optischer Fixation erhöht. Diese Resultate zeigen dass die optisch Fixation ein wichtige Rolle in der vestibulären Habituation spi lt und machen damit ine Widererwägung der üblichen Ansichten über den nervösen Mechanismus der Habituation notwendig.

be expected from the results of earlier studies, nystagmus was strongly suppressed with eyes open by contrast however quite vigorous nystagmus appeared with eyes closed Furthermore at the end of the pirouette his subjects had no difficulty in walking with eyes open, but experienced marked disorientation with their eyes closed In addition, both caloric and rotational tests carried out upon his subjects with eyes open in darkness induced enhanced nystagmic responses not present with optic fixation. The close correspondence of Collins results upon ice skaters and our own upon ballet dancers calls for special comment.

In pirouettes, ice skaters attain extremely high angular velocities of the order of 250–300 rpm and during this time the head is usually maintained in rigid conformity with the body Ballet dancers, however as is well known, invariably fix their gaze to a particular object and maintain their heads in a fixed position in space while the body rotates At each complete revolution the head is rapidly rotated and the object re fixated It is commonly believed that this manoeuvre eliminates the post rotatory stimulus that would otherwise result and consequently accounts for the absence of vertigo The theoretical considerations underlying this assumption cannot be questioned. On the other hand, if the practice conformed to the theory ballet dancers should not habituate since they would never experience unphysiological rotational stimuli Since, as has been shown, habituation in these subjects only occurs in the presence of optic fixation a more likely explanation is that ballet dancers have developed a subconscious awareness of the fact that the suppression of their vertigo is in some way dependent upon optic fixation and use it accordingly to good effect

This specificity of the phenomenon to the participation of optic fixation is in accord with the recent experimental observations of Marshall & Brown (1967) These authors showed that when normal individuals were subjected to repeated rotational tests in darkness there occurred a marked decline in the resultant nystagmic responses yet when the test stimulus was repeated with vision permitted no decline was apparent. In other words, the development of habituation in their subjects was specific to darkness. Our own observations clearly indicate that the same specificity and non transference of habituation occurs in the presence of optic fixation

As to the theoretical implications of these findings the phenomenon that we have observed is almost certainly central in origin and by analogy with recent pharmacological and electro-physiological experiments upon animals may possibly be associated, with certain presynaptic inhibitory mechanisms located in the pontine medullary portion of the reticular formation Beyond this little more can be said at the moment Whether the phenomenon we have observed can truly be described as habituation as defined by Thorpe is open to question Collins (1968) as a result of his comparable studies upon ice skaters questions the use of this term and prefers to regard the phenomenon as a modification in form of the vestibular responses rather than a simple "dropping out

In any event our studies would appear to possess an immediate and practical application to clinical neuro-otology

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RÉSUMÉ

Des épreuves rotatoires ec des danseurs de ballet comme sujet ont m ntrées, que leur seuil nystagmique à l' ccélération angulaire est normal, quand il est déterminé dans l'obscurité, mais qu'il est onsidérablement élevé en présen d'une fixation optique Ces résultats indiquent que la fixati optique joue un rôle important dans l'habituation vestibulaire et demand t pour ett raiso une réexamination des vues courantes concernant l mécanism nerveu d l'habituation vestibulaire

ZUSAMMENFASSUNG

Drehprüfung mit Balletttänzern zeigt ei en normalen Schwellenwert für den Beschleunigungsnystagmus in Dunk lheit im Gegensatz dazu ist der Schwellenwert mit optischer Fixation erhöht. Diese Resultate zeigen, dass di optisch Fixation ei wichtige Rolle in der estibulären Habituati n spielt und ma hen damit la Widererwägung der üblichen Ansichten über d n nervösen Mechanism der Habituation notwendig.

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DISCUSSION

A. Laskiewic: Twenty five subjects submitted for repeated caloric and rotatory accelerating tests, 12 figure skaters, 10 ballet dancers, and three cases of post-traumatic head sequels, have shown a clear response decline which was considerably influenced by optic fixation and was more significant with increased speed of turning or caloric stimuli. In three post-traumatic cases the habituation from vestibular responses, in which slow phase reached a distinct subnormal amplitude, was considerably weak marked. Postmortem examination of the brain in one of the named cases of traumatic injury of the occipital region has shown multilocular extravasations within the pontine part of the brainstem, including the reticular formation. This in particular seems to confirm the theoretical considerations on dependance of the cochleo-vestibular habituation from the normal function of the pontine segment of reticular formation.

A. Montandon: In order to have a scale of comparison may I ask according to what definition of the vestibular nystagmic threshold your determination was done?

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ment des globes oculaires fixant un objet exécutant des mouvements pendulaires et par stimulation avec rotation cervicale angulaire la tête étant maintenue immobile dans l'obscurité pour éliminer la fixation visuelle

L'interprétation de l'ensemble de ces investigations apporte des renseignements cliniques extrêmement utiles au diagnostic.

M Portmann La communicatio de Miss Dix et Mr Hood est très intéressante. J voudrai rappeler l'importa ce des stimulations intenses répétitive pour la durée des sejours. L u de nos collaborateurs Mr Beauchamp a (en 1960) et dié le *pelogramme* (sensation et électronystagmographie) de pilotes de jet de l'armée d l air il a constaté après un certain nombre d'heures de vol l'inhibition considérable de la réponse subjective (sensation rotatoire) alors que la réponse)stagmique était presque le même. Ce type d'adaptation portant exclusivement sur la sensation et on sur le mécanisme vestibulaire lui même est bien n phén mène purement central. Il doit être également pris en con idération.

L. B W Jongkees Ballet dancers may fixate duri g their performances. Dance ing derwishes, however have an I ward look i.e., they do not fixate. Nevertheless, it shows perfect habituation.

On ther point gives me problems. Mr Hood showed us that the caloric test, in subject with closed eyes, gives more information about the peripheral and in the subject with open eves tells u more about the central estibular ystem. How then can he use the Fitzgerald-Hallpike diagram (based on tests in patients with open eyes) to diagnose canal paresis ?

S Khechinashvili Mr Hood was ery careful when he stated that he is not quite sure whether thi phenomenon may be defined as habituation. I think that it is on of the various manifestations of the most complicated process of vestibular habituati n and if we want t speak about its central mechanism, then in the first lin central inhibition should be considered. Duri g the last decades nystagmography has given us the opportunity t st dy an entirely new phenomenon—the nystagmi eye movements occurri g i darkness and behind closed eye lids. This technique gi es the opportunity t reveal also new aspects of habituation and generally speaki g of all compensatory phenomena going n in the estibula system. Of course these two conditi ns, i.e. examination in darkness d with closed eyelids, are not identical because in the second case the involvement of sleep mechanism is more probable It is of interest to know whether the finding in darkness nd with closed evelids were the same If there was any difference then the possibility t activation of sleep or other central mecha isms should be considered.

C F Pfaltz I can confirm the hypothesis of Miss Dix and M Hood that opti f x tion i one of the most important f ctors involved in estibular habituation. This central phenomenon can lso be evoked by repeated caloric stimulations but only if the stimuli are pplied *sinusoidally* and *with* optic fixation

J D Hood (Reply) t M *Laskiewic*— Hernandez-Péon & Brust Carmona have shown that in cat habituation is unaffected by lesions transecting th brai stem at the mid-collicula level, indicati g th lower brain stem as the probable source of i hibitory mechanisms. How far however the results of these and similar animal experiment can be pplied to man is an open question, since there appear t be certai important differences between the development f the response de I ne in man and in nimals.

To M *Monland* n The method we have used t determi the threshold sensi-

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DISCUSSION

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EVOKED RESPONSES IN THE INFERIOR COLLICULUS, MEDIAL GENICULATE BODY AND AUDITORY CORTEX BY SINGLE AND DOUBLE CLICKS IN CATS

A preliminary report

B. ETHOLM

From the Institute of Neurophysiology University of Oslo Oslo Norway

This investigation shows that the auditory system has a high fidelity of the signal transmission. Using double clicks inhibitory processes were found lasting up to 150 msec and more in the medial geniculate body and the auditory cortex. In the inferior colliculus, inhibition lasted approximately 50 msec.

According to the travelling wave theory of Békésy (1947) a tone will activate a large part of the basilar membrane of the cochlea. The location of the area of maximum deflection depends upon the frequency of the tone. Correspondingly Katsuki *et al* (1958) have found that single neurones in the lower region of the auditory system respond to tone bursts of a large frequency range. In the medial geniculate body (MG) however they found a much narrower frequency band to activate individual cells (Katsuki *et al* 1959). They concluded that the analysis of the pitch of a complex sound is accomplished here. Because of the reduced active frequency range, it is likely that inhibition is involved in this analysing process.

It is the purpose of the present study to evaluate the inhibitory process at various stations of the auditory system and to see whether any inhibitory mechanisms in the medial geniculate body may explain the narrow frequency capable of driving individual MG cells.

METHODS

Cats were anaesthetized with sodium pentobarbital (30 mg/kg). Clicks of short duration were delivered to one ear through one branch of a hollow Y-formed bar inserted into the external auditory meatus. The other branch was equally long and connected to a microphone. The microphone signals were amplified and displayed on an oscilloscope, giving the strength and timing of the click. After removal of the skull, the middle portions of the lateral suprasylvian and ectosylvian gyri were removed by suction. After

tivity of the semi-circular canals is entirely empirical. It does, however possess the advantage of simplicity being dependent upon the detection of the absence or presence of a response, and in this respect is not dissimilar to conventional methods for the determination of the threshold of hearing, insofar as the liminal response may be bracketed between stimuli evoking a clear nystagmic response and stimuli evoking no response.

To Mr *Greiner* and Mr *Pfalt* It is extremely interesting that habituation does not appear to occur from pendular stimulation. The reason for this is difficult to explain. It may be that the resultant constantly changing stimulus does not comprise the monotony which seems to be one of the necessary features for the development of habituation Our own studies have been confined to subjects already habituated and we have carried out no systematic series of investigations upon normal subjects. The results of Marshall & Brown (1967) however would seem to suggest that habituation is specific to the stimulus conditions.

To Mr *Portmann* Mr Portmann's comments are extremely interesting and are in parallel with our own findings. They stress the need for careful enquiry into flying sea-going experience or any other occupation that might give rise to habituation in all patients found to have bilateral abolition or reduction of the caloric responses.

To Mr *Jongkees* The absence of vertigo in dancing derwishes may in part be explained by the fact that they rotate for very long periods at constant velocity However as Mr Jongkees so rightly implies, movement of the head during this time must stimulate the vertical canals and one can only assume that these responses, too habituate though I am not aware of any studies specifically directed towards habituation resulting from stimulation of the vertical canals. With respect to canal paresis a bilateral paresis is a total abolition of the responses, as for example in streptomycin poisoning and in such cases there is, of course absence or reduction of the rotational responses both in the presence and absence of optic fixation

Rotational tests, however have limited localising value, and it is difficult to see how they can provide any information upon unilateral canal paresis since both sets of canals are stimulated simultaneously

To Mr *Khechinashvili* I do not think it is possible at this time to say with any certainty that the phenomenon we have studied is true habituation. In any event it does not seem to be vestibular suppression a term often used nowadays to describe the phenomenon in the sense that the end organ response is centrally inhibited. The question of sleep mechanisms complicating the interpretation of electro-nystagmographic recordings is extremely important. In this respect however recording in darkness with the eyes open and recording behind closed lids are not the same. Induced nystagmus recorded with eyes closed is much more likely to be influenced by environmental factors and by mental activity than when recordings are carried out with eyes open in darkness, and it seems not unreasonable to suppose that it is not the removal of vision but the act of closing the eyes which initiates sleep mechanisms, mediated I suppose by way of the reticular formation

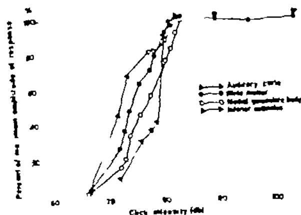


FIG. 2. Click-evoked responses in per cent of maximum amplitude f response from the inferior colliculus, the medial geniculat body the white matter nd the uditory cortex.

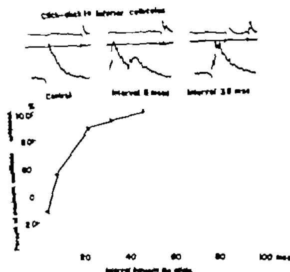


FIG. 3. The effectl eaem of conditioning click on the response t test click recorded from the I ferior colliculus. V riable interval between the two clicks. Both clicks had h gh tensity

2 shows the relation between the strength of the click and the size of the responses obtained at the four stations, expressed as the percent of the maximum amplitude of the responses. The evoked potentials increased from 0 to 100 per cent over a range of click intensity of only 15 dB. The input–output curve was steepest for the responses of the colliculus inferior then followed the curves for the white matter and the medial geniculate responses, and at last, the auditory cortical responses.

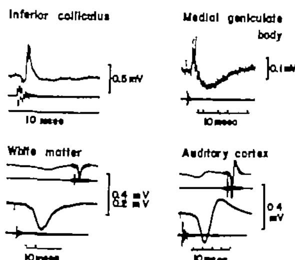


FIG 1 Typical click-ev ked responses recorded from the inferi r colliculus, th medial geniculate body the white matt and the auditory cort x.

exposure of the lateral ventricle, the fimbria and the hippocampus were removed with a glass seeker thus exposing the dorsal surface of the thalamus and the superior and inferior colliculi

The recording electrodes were introduced through small holes in the pia on the dorsal surfaces of the thalamus and the inferior colliculus, respectively Recordings from the inferior colliculus and the medial geniculate body were made with extracellular glass microelectrodes, whereas the white matter and cortical records were made with 0 2 mm thick platinum electrodes, insulated except at the tip

RESULTS

The first figure shows typical recordings from the inferior colliculus, the medial geniculate body the auditory radiation (white matter) and the auditory cortex in response to a click of high intensity In all records negativity is upwards The second line in all records represents the microphone deflection signalling the intensity and the onset of the click at the eardrum In the response from the colliculus inferior there was first a little positive wave, followed by a large negative wave with superimposed spikes representing the firing of the cells. The negative wave was usually followed by a positive wave although not distinct in this particular recording In the recording from MG the sweep speed was higher The response had almost the same configuration as in the inferior colliculus with an initial positive wave followed by a larger negative wave with spikes However the late positive wave was more distinct and had a longer duration In the white matter record, the response was a pure positive wave With a surface electrode on the auditory cortex, the click evoked potential was the ordinary positive/negative diphasic primary evoked potential

The auditory system shows a high fidelity of the signal transmission Fig

RÉSUMÉ

Les recherches présentées indiquent une transmission nerveuse de haute fidélité dans le système auditif. En utilisant des clicks doubles, on a trouvé des méchanismes inhibiteurs durant jusqu'à 150 milli-secondes dans le corpus geniculatum médiale et le cortex auditif. Dans le colliculus inferior l'inhibition a duré environ 50 milli-secondes.

ZUSAMMENFASSUNG

Die Untersuchung zeigt, dass Lautstimuli in dem auditorischen System leicht passieren. Durch Anwendung von doppelten Klicken hat man inhibitorische Phänomene gefunden die 150 Millisekunden oder mehr dauerten, sowohl in Corpus geniculatum mediale als im uditorischem Cortex. In Colliculus inferior dauerte die Inhibition ungefähr 50 Millisekunden.

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DISCUSSION

K. Burian: Zu den sehr interessanten Ausführungen von Hr. Elholm möchte ich meine eigene Erfahrung aus der klinischen EEG-Computer-Audiometrie hinzufügen. Bei Kindern mit abnormen EEG gelingt es mitunter nicht die akustisch evozierten Potentiale bei schwellennaher Stimulierung nachzuweisen. In solchen Fallen haben wir das Phanomen der Interaktion zweier akustischer Reize angewandt, das im Prinzip den Beobachtungen von Hr. Elholm entspricht. Dabei bietet man nach dem fraglichen, schwellennahen Prüfton mit einer Latenz von 120–150 msek einen zweiten sehr lauten Ton an, der sicher erkennbare Potentiale evoziert. Im Falle der erste schwellennahe Stimulus tatsächlich perzipiert wurde treten die beiden Potentiale in Interaktion. Dadurch wird die evozierte Antwort auf den Zweitreiz in signifikanter Weise verändert. Aus dieser Veränderung kann man auf die tatsächliche Perzeption des schwellennahen Erstreizes rückschliessen.

B. Elholm (Reply) to *M. Burian:* Mr Burian presented some very interesting results demonstrating that a stimulus with no measureable response still may have an inhibitory effect. I did not notice any time scale on the slide presented and wonder what the interval is between the two tones. In the future we have plans to do similar investigations.

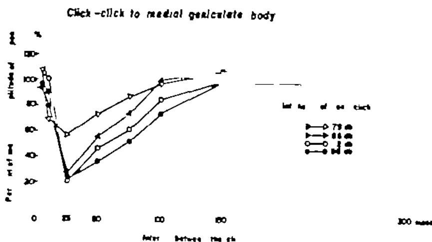


FIG 7 Recordings from the medial geniculate body sh wing how the inhibition was dependent upon the i t nsity of the conditioning click.

culate body and further to the auditory cortex. All responses are normalized by setting the maximum response to 100 per cent In the inferior colliculus, the inhibition was moderate There was a great difference as the volley passed through the medial geniculate body The added depression of the medial geniculate responses and of the recordings taken from the white matter indicates a marked inhibition of the medial geniculate neurones. In the white matter the inhibition lasted up to 150–200 msec. The condition ing click inhibited the response to the test click completely with an interval between the two clicks up to 100 msec The recordings from the surface of the cortex were similar to the recordings from the white matter

The inhibition is dependent upon the strength of the conditioning click Fig 6 shows responses from the inferior colliculus. Conditioning click in tensities of 65 and 70 dB gave no inhibition. To the contrary at an interval of 10 msec there was an increased response due to a summation of excita tory postsynaptic effects of the two clicks. With stronger shocks, inhibition occurred, the major part of the inhibition lasting only 25 msec.

In contrast to this behaviour Fig 7 shows responses from the medial geniculate body A conditioning click of an intensity of 79 dB produced a moderate inhibition With increasing intensities of the conditioning click both the strength and duration of the inhibition were more pronounced

The same phenomenon was seen when the recordings were made from the white matter Here the inhibition was more pronounced than in the medial geniculate body The auditory cortex showed similar responses.

The investigation has shown that there exists inhibitory mechanisms in the upper part of the auditory pathway In the inferior colliculus, the inhibi tion is moderate and lasts only up to 50 msec In the medial geniculate body the inhibition is stronger and has a longer duration. It is likely that the inhibition in the medial geniculate body is of both pre- and postsynaptic nature

observers may need 60–90 μsec. The instrument is connected to an audiometer via an interrupter which produces short tone bursts. The frequency of the tone can be selected on the audiometer The interrupter unfortunately produces also a sharp click, being a more predominant cue for lateralization than the pure tone which follows. By using the click-free switch of the audiometer a transient free tone burst can be presented to the listener's ears. It appears that normal listeners are able to lateralize for time (=phase) differences with the aforementioned precision in the frequency range from low (e.g 125 Hz) to about 800 Hz, beyond which frequency this ability rapidly declines. If the click is introduced lateralization is possible up to 4000 Hz although the precision is less than in the lower tone scale The Mediophon guarantees the constancy of the intensities in the two headphones whatever time delay is introduced. This is extremely important for the following reason

We have been using this instrument for several years on different kinds of patients, amongst whom were cases with disorders of the acoustic nerve, e.g. acoustic neurinoma It appeared that in the latter group Δt discrimination was generally absent whereas interaural intensity differences ΔI of normal magnitude (2 dB) already produced lateralization

So, if we intend to investigate retrocochlear dysfunction, we should maintain a constant sound level but vary the interaural time delay Δt which is the most sensitive criterion for neural disorders.

In directional hearing both parameters phase (=time) difference Δt and intensity difference ΔI play important roles. Even as low as 500 Hz a 2 dB intensity difference is attained already at an azimuth of 12° the higher the frequency the stronger the shadow effect of the head and the more the ΔI cues will contribute to localisation. Nordlund (1963) worked with a loudspeaker in a free field, using pure tones in the range from 500–8000 Hz. Also bursts of noise (filtered) served as test sounds. We must assume that his patients had the benefit of both parameters. This may have been the reason that directional hearing was only impaired in cases of acoustic neurinoma but not abolished, because the interaural intensity differences ΔI helped these patients to locat the sound source

The ability to detect interaural time differences is highly developed in normal subjects as has been stated previously It is worth while to go into this matte somewhat deeper

If two identical sound signals are presented to the two ears almost simultaneously each cochlea will generate a volley group with a certain time pattern This message travels via the acoustic nuclei to the accessory nucleus of the oliva superior where it will meet its exact duplicate coming from the other cochlea The conducting time from cochlea to live is of the order of 3 milliseconds. Yet during this relatively long time, the time pattern of the volley group appears to be preserved with a precision better than 1% for 30 μsec interaural time delay is already noticed and interpreted by the listener a ju t beside the midline From this point of view it is plausible

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DIAGNOSTIC VALUE OF LATERALIZATION ABILITY FOR DICHOTIC TIME DIFFERENCES

J J GROEN

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The ability for lateralization of two sound signals, identical in all aspects except for time of arrival in the two ears, is an important diagnostic tool in the differential diagnosis between cochlear and retrocochlear deafness. A simple instrument for the lateralization test will be introduced and the results discussed.

The differential diagnosis between cochlear and retrocochlear dysfunction has gained considerably in reliability during the last 20 years. Nowadays a whole battery of audiometric tests helps us to localize the site of the lesion in the auditory pathway Perceptive deafness of cochlear origin is relatively easy to diagnose recruitment(and its allied phenomena) is the decisive symptom

Less conclusive are the criteria for retrocochlear deafness. There is one exception Different authors all agree upon type III of Jerger s classification if the curves for interrupted and continuous tone presentation diverge strongly a serious disturbance in the acoustic nerve is most probable Slighter dysfunction of the acoustic nerve, however may escape notice

In the past several authors have recommended the use of directional hearing for the diagnosis of central auditory disturbances In particular brain tumors in the temporal lobe would impair the precision in locating a sound source Greene (1929) used a buzzing sound source in free field conditions and presented it also through a stethoscope His results were that the ability to locate the source in free field or to lateralize it in stethoscopic presentation was significantly impaired in patients with tumors of the temporal lobe Of the same opinion were Sanchez Longo & Forster (1957 1958) who worked with the telephone receiver of a Maico audiometer the latter switched to the position "masking thus producing a harmonic complex consisting of multiples of 60 Hz.

Matzker & Welker (1959) described an instrument (Medlophon) which contains a variable delay line, enabling the investigator to introduce an adjustable interaural time difference Δt between otherwise identical signals Δt can be regulated stepwise from 36 μsec up to 648 μsec. Normal listeners start to lateralize this dichotic sound presentation as soon as a time difference of about 30 μsec is reached none too-careful but otherwise normal

speech discrimination score was poor 20% at the best. Loss of coherence must have been caused here by too great a dispersion of conduction velocity in the neurons.

In all cases of perceptive deafness examined, we have encountered Δt discrimination inability only in n VIII disturbances this is in agreement with the findings of Nordlund (1963) Unilateral disorders in the temporal lobe never abolish Δt perception, although the minimum Δt values may be increased slightly This is also in agreement with Nordlund s data, but is in contrast to the opinion of Sanchez Longo & Forster (1957)

The success of the Δt test has led to its frequent use in our audiology department—to such an extent that it would be worth while to look for a simpler form, easy to operate and manufacture. It should, however also fulfill the condition of constancy of sound level when interaural time difference is varied. A modified version of the stethoscope (see also Greene) was chosen (Groen, 1967) A rubber tube of 130 cm length, outer diameter 10 mm, inner diameter 7 mm, is connected to the tubes of a normal stethoscope frame. Over a distance of 15 cm to the left and right of the rubber center it is enclosed in a brass tube from which almost one half is removed, so that the rubber will slightly bulge over the edges of its brass housing. A frame with grip connects center and ends of this housing which also is engraved with cm markings to left and right of tube center The housing with grip is necessary in order to prevent the generation of highly disturbing contact noise the bare rubber tube certainly would produce even when handled with care (Fig. 2)

The test subject puts the earpieces of the stethoscope into his ears. The tube is very gently tapped by a leadpencil or similar implement at, e.g., 10 cm distance from tube center The test subject should be able to lateralize thi presentation in the nearer ear If he does, the tube is tapped on the other side somewhat nearer to tube center Alternating left and right tapping and closing in on center the smallest distance from center is determined which just gives rise to a lateralization. If the ears of the patient are different a t hearing loss, midline localisation will occur away from tube center towards the poorer ear This is explained by the time-intensity trade function of David *et al* (1958) a difference in loudness can be compensated for by a certain time-difference The poorer ear receives the message first and converts it into a neural signal. This will encounter longer synaptic delays than its stronger counterpart from the other ear and will arrive at the same time a this ne in the olive The uncertainty in Δt around patient s center" hould, however be the same as in normal listeners.

The tap n the tube wall starts off two travelling waves which have the same time pattern and sound spectrum, which, in our tube shows maxima at about 200 400, and 600 Hz. Beyond 600 Hz the spectral envelope declines rapidly (Fig 3) After about 2 msec the travelling waves will have changed nto tanding waves because of the finite length of the tube and the reflec tion gain t the closed endings. Difference in attenuation of the waves is

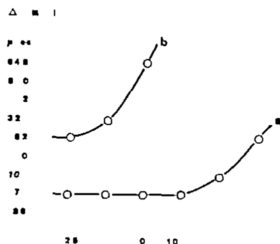


Fig 1

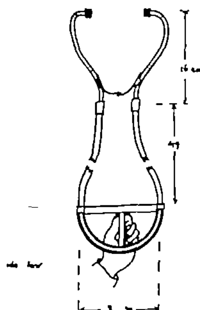


Fig 2.

Fig 1 (*a*) Averag Medi ph n (with lick) Δt data on a group of children with perceptive deafness f cochlear origin hearing loss between 40 and 85 dB n both ears (Fletcher average) Tube Δt dat 66 μsec. (*b*) Average Mediophon (with click) Δt data on a group of children with perceptive deafness of retrocochlea origin (kernicterus) hearing loss between 35 and 65 dB (Fletcher average) Tube Δt data: from 400 μsec to u measurable

Fig. 2. Tube Δt meter

that even a minor disturbance in conducting properties of the nerve will endanger time pattern conservation and thus will reduce the ability for interaural time difference discrimination This ability is not directly related to the pure tone audiogram (Nordlund) We found in a group of children (average age 12 years) with perinatal anoxia, having hearing losses ranging from 40 to 85 dB (Fletcher average) in both ears, that Δt values varied from 60 to 90 μsec (thus in the normal range) In contrast to this, another group of children (average age also 12 years) with kernicterus at birth (due to Rhesus incompatibility of the parents) all having a sloping audiogram with a moderate loss of 45 dB (Fletcher average) yielded Δt values ranging from 400 μsec to infinite (unmeasurable) (Fig 1) A unilateral acoustic tumor even when pure tone hearing loss was moderate (e g 35 dB in the whole frequency range) led to complete abolition of interaural time difference discrimination These patients keep hearing two separate sound impressions which will not fuse into a lateralized sound image. It appears that coherence in time pattern is lost when the signal is transported along the affected nerve rendering impossible the fusion with the signal coming from the other side into the olive When asked how speech sounded through the affected ear as compared with the other one (normal) they said it sounded confused, echoic, as when spoken in a highly reverberating room Their

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In all cases of perceptive deafness examined, we have encountered Δt discrimination inability only in n VIII disturbances this is in agreement with the findings of Nordlund (1963) Unilateral disorders in the temporal lobe never abolish Δt perception, although the minimum Δt values may be increased slightly This is also in agreement with Nordlund's data, but is in contrast to the opinion of Sanchez Longo & Forster (1957)

The success of the Δt test has led to its frequent use in our audiology department—to such an extent that it would be worth while to look for a simpler form, easy to operate and manufacture. It should however also fulfill the condition of constancy of sound level when interaural time difference is varied. A modified version of the stethoscope (see also Greene) was chosen (Groen, 1907) A rubber tube of 130 cm length, outer diameter 10 mm, inner diameter 7 mm, is connected to the tubes of a normal stethoscope frame Over a distance of 15 cm to the left and right of the rubber center it is enclosed in a brass tube from which almost one half is removed, so that the rubber will slightly bulge over the edges of its brass housing A frame with grip connects center and ends of this housing which also is engraved with cm markings to left and right of tube center The housing with grip is necessary in order to prevent the generation of highly disturbing contact noise the bare rubber tube certainly would produce even when handled with care (Fig 2)

The test subject puts the earpieces of the stethoscope into his ears. The tube is very gently tapped by a leadpencil or similar implement at, e.g 10 cm distance from tube center The test subject should be able to lateralize this presentation in the nearer ear If he does, the tube is tapped on the other side somewhat nearer to tube center Alternating left and right tapping and closing in on center the smallest distance from center is determined which just gives rise to a lateralization. If the ears of the patient are different as to hearing loss, midline localisati n will occur away from tube center towards the poorer ear This is explained by the time-intensity trade function of David *et al* (1958) a difference in loudness can be compensated for by a certain time-difference The poorer ear receives the message first and converts it into a neural signal. This will encounter longer synaptic delays than it tronger counterpart from the other ear and will arrive at the same time a this one in the olive. The uncertainty in Δt around patient's center hould, however be the same a in normal listeners.

The tap on the tube wall tarts off two travelling waves which have the same time pattern and sound pectrum, which, in our tube shows maxima at about 200, 400 nd 600 Hz. Beyond 600 H the spectral envelope declines rapidly (Fig 3) After about 2 msec the travelling waves will have changed int tand g waves because f the finite length of the tube and the reflec tion ga n t the closed endings. Difference in attenuation of the w ves is

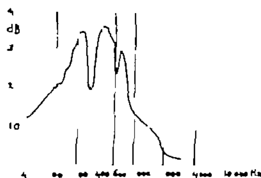


Fig 3 Energy distribution as a functi n of frequency of a "tap" n the rubber tube

negligible even for tapping at the extreme point near the end of the brass housing (15 cm from tube center) (Norton, 1935 Fay 1940) If a bone conduction receiver is used in contact with the tube, lateralization occurs *due to phase differences* depending on the distance between contact point and tube center This method has been used by van Soest & Groot (1929) it is a variation on the original work of *Hornbostel & Wertheimer* (1920) In the low frequency range the bone conduction receiver connected to an audiometer will give detailed information per frequency as to patient s ability to detect phase differences. As has been stated previously the tap produces a complex sound the reactions of the patient then will pertain to his potentialities in the whole lower frequency range (up to 600 Hz) which is good enough for clinical purposes.

The margin of error of the normal listener is about 1 cm on the tube tapping should then take place at 0 5 cm from tube center (the distance to the nearer ear is $2 \times 0.5 = 1.0$ cm shorter than to the farther ear) As the velocity of sound in the tube is practically 340 m/sec, the corresponding time difference then will be about 30 μsec, which is the minimum delay normal listeners can detect The average untrained listener will need some more, so 1 0 or 1.5 cm around tube center should be considered as a normal margin of error 3 cm is already pathological

In this way the Δt meter provides us with an easy method to detect abnor *malities in 1st and/or 2nd order neurons. The instrument is easy to operate* the whole test takes about 1 minute even a child can undergo it Tube and Mediophon data obtained on different kinds of patients, are consistent Whenever a patient fails to lateralize even with the maximum Mediophon delay (648 μsec) he will not lateralize either with the tube and vice versa Also the exact Mediophon delay times can be reproduced with the tube in terms of centimeters distance around the patient s center at the tube Purely *cochlear lesions do not disturb Δt-detection middle ear abnormalities* show normal Δt values, be it around a new midpoint shifted away from tube center in the direction of the worse ear

RÉSUMÉ

Le pouvoir de latéraliser des signaux binauraux identiques en tous aspects, e cepté pour le moment d arrivée dans les deux oreilles, nous offre une méthode de diagnostique différentiel important entre surdité de perception cochléaire et rétrocochléaire. U instrument simple pour ce test de latéralisation sera introduit et les résultats seront discutés.

ZUSAMMENFASSUNG

Das Lateralisati svermögen für binaurale Signale, identisch in allen Hin sichten, nur verschieden in den Momenten der Ankunft in beiden Ohren ist ein werti Des Messverfahren für die Differentialdiagnose zwischen kochleärer und retrokochleärer Schwerhörigkeit. Ein einf hes Instrument für die Lateralisationsuntersuchung wird introduziert und die Ergebnisse diskutiert.

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DISCUSSION

G Lidé There has been mu h confusion concerni g the diagnosti value of sound local zat on tests. Therefore it was very interesti g t hear that M Groen has been bl t confirm the result that Mr Nordlund in our laboratory has earlier presented with a free field sound localization test. As Mr Groen, we have found the sound localization test to be ery sensiti for detecting early lesions in th e ghth nerve

J E Bordley Thi i a most important paper which Mr Groen has presented i his usual excellent man er His observation concerni g the possibility of the

delayed conduction time of the eighth nerve underlying this difficulty in localizing the direction of sound has been confirmed in another way by Shinizer in our laboratory Timing the appearance of cortical potentials evoked by a sound stimulus, he has found an increased conduction time in the case of eighth nerve tumours. He has also found a shortened conduction time in cases of Menière's disease We have not tested these cases for sound localization, but our findings would suggest that the shortened conduction might also result in difficulty in tone localization If this is true we might have two different lesions causing localizing difficulty

J J Groen (Reply) to Mr *Lidén* I am glad to learn that he is still of the same opinion as I am

To Mr *Bordley* I want to thank Mr Bordley for his comments, especially on the work from his laboratory where EEG latency problems were studied in cases with peripheral tumors and in cases of Menière

Whereas we find normal Δt values in Menières patients, he finds shorter latencies than in normals. This discrepancy may perhaps be attributed to the difference in level on which these phenomena occur in EEG audiometry these are cortical and in Δt-measurements they are on the level of the olivary complex

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EEG-COMPUTER-ANALYSE — SINNLOSER UND SINNVOLLER AKUSTISCHER REIZE

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Bei normalhörenden Versuchspersonen wurde eine vom Prüfling un abhängige Objektivierung des akustischen Diskriminationsvermögens angestrebt. Bei wiederholter Anbietung sinnloser oder innvoller Worte mit nachfolgendem Lichtreiz konnt ei e Erwartungswelle im Computer-gemittelten EEG achgewiese werden Dabei stellt das dem Licht blitz vorangehende Wort einen Warnreiz dar der die Ausbildung eine Erwartungsw lle vor dem Lichtreiz Signal ermöglicht. Solche Erwar tungswellen könn n selekti durch di Kombination sinnloser oder sinn voller Wort mit inem Lichtreiz ausgelöst werden N chdem ein der artige Sel kti n nur möglich ist, w n die Versuchsperson den Bedeutungsgehalt der Testworte differenziert, ergibt sich daraus ein objek tiver Hinwei a f deren Diskrimination vermögen.

Die EEG-Computer-Audi metrie (ECA) vermittelt objektive Hinweise auf die akustische Perzeption, Reizleitung und Reizausbreitung im Cortex. Der Nachweis akustisch evozierter Potentiale sagt jedoch nichts über die gnostische Verarbeitung und Diskrimination der akustischen Signale aus. So kann es beispielsweise vorkommen, dass man bei Kindern, die als hochgradig schwerhörig gelten und auch typische Symptome dafür aufweisen, im ECA eine normale Tonhörschwelle registriert. Es kann sich dabei um eine Aphasie oder psychische Hörstörung handeln, die mit der bisher üblichen Technik der Click oder Sinustonstimulierung nicht objektiviert werden kann Ebenso wie in der subjekti n Audiometrie erst die Anwendung der Spra haudiometrie die Beurt ilung des Gesamthörvermögens ermöglicht hat, waren auch für die EEG-Computer Audiometrie Methoden erforder lich, die ein obj ktive und von der Mitarbeit des Patienten unabhängige Beurteilung des Sprach erstandnisses und der Diskrimination gestatten Einen ersten Beitrag zu diesem Problem stellen die folgenden Versuche dar

Trotz der sehr umfangreichen Literatur über sensorisch evozierte Potentiale gibt es relati wenig Arbeiten, die sich mit dem Problem der Diskriminierung sensorischer Reize befassen. *Oswald et al* (1960) konnten bei V rsu h personen während bestimmter Schlafstadien nach Anbietung ver t ndlicher W rtserien wesentlich häufiger K-Komplexe im EEG beobachten al n ch aku tischen Aequivalenten (Vor oder Rückwärtssprechen be

kannter Vornamen) Bei Reizsituationen unterschiedlichen Informationsgehaltes haben Sutton *et al* (1967) an Hand gemittelter evozierter Potentiale positive Potentialschwankungen beobachtet Diese traten erst zu einem Zeitpunkt auf da das Reizgeschehen mit Sicherheit erkannt wurde. Diese Positivierung des EEG-Verlaufes dürfte möglicherweise auf einen Abfall der langsamen negativen Potentialabweichung zurückzuführen sein, die durch Bereitschafts- Erwartungs- oder Orientierungsreaktionen ausgelöst wird. In diesem Zusammenhang sei auf eine Arbeit von Caspers (1961) verwiesen der corticale Gleichspannungsschwankungen bei Ratten beobachtete, die bereits vor bestimmten Bewegungen vor allem Orientierungsbewegungen auftraten und als Ausdruck der Bereitschaft bzw Erwartung einer motorischen Handlung aufzufassen sind Kornhuber & Deecke (1965) konnten solche Potentialschwankungen vor Willkürbewegungen auch beim Menschen nachweisen und bezeichneten sie als „motorische Bereitschaftspotentiale Gilden *et al* (1966) haben Gleichartiges beobachtet und als motor potential bezeichnet Walter *et al* (1964) beschrieben unter der Bezeichnung Contingent Negative Variation" oder „Erwartungswelle eine langsame, oberflachen negativ gerichtete Potentialänderung, die zwischen einem Warn und Signalreiz mit nachfolgender motorischer Reaktion auftrat Nach unerwarteten Zwischenreizen mit nachfolgender motorischer Reaktion welche in regelmässige Reizserien eingestreut waren, beschrieben Haider *et al* (1968) ähnliche negative Wellen Diese als Orientierungspotentiale bezeichneten Wellen konnten auch dann beobachtet werden, wenn nach dem Zwischenreiz keine motorische Reaktion folgte.

Die zitierten Literaturangaben lassen erkennen, dass es unter einer entsprechenden Versuchsanordnung möglich wäre langsame Hirnpotentialänderungen welche mit Erwartungsvorgangen verknüpft sind als elektrophysiologische Kriterien der Diskriminationsleistung heranzuziehen

METHODIK

In drei verschiedenen Tests wurden insgesamt 70 Versuchspersonen 60 mal zufallsverteilt verschiedene Worte angeboten In der ersten Versuchsreihe war es nur ein sinnvolles und leicht verstandliches Wort (Mama) und ein sinnloses, phonetisch annaherndes Äquivalent (Fama) In einem zweiten Versuch wurden 8 sinnvolle Worte und 8 sinnlos phonetische Äquivalente mit der gleichen Zufallsverteilung angeboten Der dritte Test schliesslich enthielt 30 sinnvolle Worte und 30 sinnlose Buchstabenkombinationen deren phonetischer Gehalt keine Korrelation zu den sinnvollen Worten aufwies Unter den gleichen Bedingungen haben wir in jeder Versuchsreihe abwechselnd das sinnvolle und das sinnlose Wort mit einem Lichtblitz kombiniert, der mit einer Verzogerung von 1270 mSec nach Beginn der Analysationszeit angeboten wurde Durch diese Reizkoppelung sollte das Zustande-

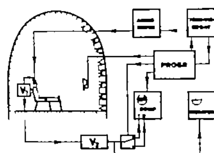


Abb. 1

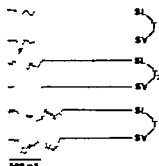


Abb. 2

Abb 1 Block Schaltbild der gesamten Anlage. V Vorverstärker; V Ha pt erstärker

Abb. 2 Summation der über Mikrophon ufgenommenen Wortreize der drei Testprogramme.

kommen einer Erwartungswelle ermöglicht werden. Schliesslich wurde noch der Einfluss der Lautstärke auf das Diskriminationsvermögen untersucht dazu wurden die Testworte vergleichsweise überschwellig (75 dB) und schwellennahe (25 bis 35 dB) angeboten. Die schwellennahe Intensität wurde individuell festgelegt und zwar derart, dass der Prüfling gerade noch alle Worte richtig nachsprechen konnte

Die Ableitungselektroden wurden mit Hilfe von Bentonit Leitpaste auf den Vertex und auf das Mastoid (Referenz) aufgeklebt. Die abgeleiteten Potentiale wurden über Vor und Hauptverstärker einer Schaltanlage zugeführt, welche die erhaltenen Informationen in zwei Kanäle eines Computers (CAT 400) aufteilte. Kanal 1 (in den Kurvenbildern oben dargestellt) analysierte das EEG während der sinnlosen und Kanal 2 (in den Kurvenbildern unten dargestellt) während der sinnvollen Wortstimulierung. Nach dem bei dieser Anordnung dieselben Elektroden und die gleichen Verstärker für die Analysation beider Wortreize verwendet werden, ist eine verlässliche V rgl ichs ituation gegeben Die Testworte wurden zufallsverteilt auf eine Spur des Tonbandes aufgesprochen, auf der zweiten Spur des Bandes wurden Schaltimpulse aufgezeichnet, die den Beginn der Worte, getrennt nach inn ll und sinnlos, einem Programmierer zuführten, welcher die Synchronisation der Computeranalyse und der Kanalwahl vornahm (Abb 1) Die Versuchsperson befand sich in einer schall- und echoarmen Kammer Die aku tischen Reize wurden durch Kopfhörer vermittelt, während in X n n Blitzgerät zu gegebener Zeit die optischen Zweitreize produzierte.

Zur Kontrolle der gesamten Apparatur wurde das Programm anstelle der Versuchsperson über ein Mikrophon aufgenommen und gemittelt. Abb. 2 zeigt die Summation der sinnlosen (SL) und sinnvollen (SV) aku tischen R ize der drei Testprogramme Die Analysati nszeit betrug 2 Sekunden, die Zeitkon t nte 1,2 Sek.

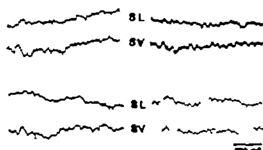


Abb. 3

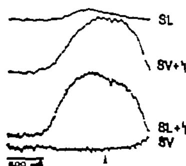


Abb. 4

Abb. 3 Vergleich der gemittelten Potentiale des Wortes Fama" (*obere Kurve*) und Mama (*untere Kurve*) bei vier verschiedenen Versuchspersonen

Abb. 4 Typische Erwartungswellen, ausgelöst durch die Kombination eines sinnvollen und eines sinnlosen Wortes mit einem Lichtblitz (*S.L.*, Sinnloser Wortreiz *SV* Sinnvoller Wortreiz)

ERGEBNISSE

Bei dem ersten Test mit Unterscheidung zwischen nur zwei Worten zeigte sich dass die evozierten Potentiale nach Anbietung des sinnvollen Wortes grosser und ausgeprägter waren als nach dem sinnlosen phonetischen Äquivalent Der Grössenunterschied wies zwar eine betrachtliche Streubreite von wenigen bis über 100% auf konnte jedoch immer erkannt werden (Abb 3)

Mit einem zusätzlichen Lichtreiz nach jedem sinnvollen Wort konnte eine langsame negative Potentialschwankung im Sinne einer Erwartungswelle ausgelöst werden Sie trat bei den meisten Fällen deutlich in Erscheinung, bei einigen wenigen Versuchspersonen waren sie nur schwach ausgebildet, aber erkennbar Diese Erwartungswelle tritt zwischen dem Wort und Lichtreiz auf Bietet man den Lichtreiz nach dem sinnlosen phonetischen Äquivalent an dann kann man gleichfalls eine Erwartungswelle beobachten wobei in dieser Versuchsanordnung das allein angebotene sinnvolle Wort (untere Kurve) als Vergleichsreiz dient (Abb 4)

In einigen Versuchen liessen wir nach dem Lichtblitz eine motorische Reaktion ausführen (Handheben) In einer solchen Differenzierungssituation verstarkt diese die Erwartungswelle nicht oder nur ganz unwesentlich (Abb 5) Unterschiede im Kurvenverlauf betreffen lediglich den Zeitpunkt der motorischen Reaktion selbst nicht jedoch die Erwartungswelle In den folgenden Versuchen haben wir daher diese Kombination nicht mehr angewandt

Bei wiederholter Anbietung von nur einem sinnvollen und einem sinnlosen Wort wäre es möglich, dass die Versuchsperson durch die Wiederholung desselben sinnlosen Wortes dieses als gleichbleibendes Phonem in Erinnerung behält und somit zwischen der phonetischen Charakteristik, nicht aber zwischen dem Bedeutungsgehalt beider Worte differenziert Wir

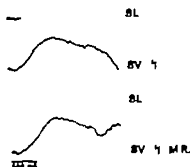


Abb. 5.

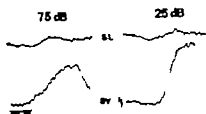


Abb. 6.

Abb. 5. Erwartungswelle bei Kombination eines sinnvollen Wortes mit einem Lichtblitz (*oberes Kurvenpaar*) und zusätzliche motorische Reaktion nach dem Lichtblitz (*unteres Kurvenpaar*)

Abb. 6. Erwartungswelle nach sinnvollem Wort und Lichtblitz bei 75 dB (*oberes Kurvenpaar*) und 25 dB (*unteres Kurvenpaar*)

haben daher in dem zweiten Versuch acht verschiedene sinnvolle Worte von acht unverständlichen phonetischen Äquivalenten unterscheiden lassen. Bei diesem Test zeigt sich der Unterschied im evozierten Potential des sinnlosen und sinnvollen Wortes nicht mehr so regelmässig und deutlich als im ersten Versuch. Bei der Kombination von sinnvollem Wort und Lichtblitz konnte jedoch wieder eine deutliche Erwartungswelle vor dem Lichtblitz nachgewiesen werden. Wurde zur Kontrolle der Lichtblitz nach dem sinnlosen Wort angeboten, so konnte nun die Erwartungswelle, ebenso wie bei dem vorangegangenen Versuch, auch durch das sinnlose Wort ausgelöst werden. Dieses Verhalten zeigt, dass die Versuchsperson den Warncharakter des Wortreizes in Hinblick auf den Lichtblitz als nachfolgendes Signal jeweils erkannt und somit zwischen sinnvollem und sinnlosem Wort unterschieden hat.

In dem dritten Versuch wurde die Differenzierungsleistung noch weiter gesteigert. Dabei wurden 30 verschiedene sinnvolle Worte und 30 sinnlose Buchstabenkombinationen zufallsverteilt angeboten, damit kommt es während des gesamten Testverlaufes zu keiner Wortwiederholung und die Versuchsperson muss den Bedeutungsgehalt jedes einzelnen Testwortes erkennen. Auch unter dieser Versuchsanordnung konnte eine typische Erwartungswelle nachgewiesen werden, wobei diese bei wechselnder Kombination des sinnvollen oder sinnlosen Wortes mit einem nachfolgenden Lichtblitz immer dort auftritt, wo der Lichtreiz angeboten wurde.

Bei Testung mit schwellennaher Intensität beobachtet man die gleichen Ergebnisse, wobei die Amplitude der Erwartungswelle oft noch grösser ist als bei überschwelliger Stimulierung (Abb. 6). Bedenkt man, dass die Versuchsperson bei schwellennahem Reiz eine wesentlich grössere Konzentration benötigt, um den Bedeutungsgehalt des angebotenen Reizes zu erfassen, dann bestätigt dies die schon mehrfach beschriebene Beobachtung, dass mit

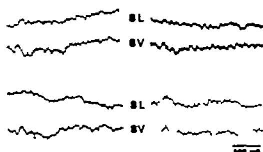


Abb. 3

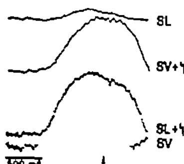


Abb. 4

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sehen zwei, acht und dreissig sinnlosen und sinnvollen Worten gezeigt werden konnte. Zur Ausbildung einer Erwartungswelle vor einem Lichtreiz ist es notwendig, dass die Versuchsperson den Warncharakter des dem Lichtsignal vorausgehenden Wortreizes erkennt. Nachdem der Lichtreiz immer nur nach einem bestimmten Reiz, nämlich einem sinnlosen oder sinnvollen Wort angeboten wird, besteht der Warncharakter des Wortreizes in seinem Bedeutungsgehalt. Eine diesbezügliche Unterscheidung setzt eine gnostische Verarbeitung und Diskriminierung beider Wortreize voraus. Bei zweikanaliger Registrierung der Potentiale von sinnlosen und sinnvollen Worten mit nachfolgendem Lichtreiz bzw. umgekehrt, stellt daher die Erwartungswelle einen Beweis für die Diskriminationsleistung der Versuchsperson dar. Dabei ist es irrelevant ob der Lichtreiz nach dem sinnlosen oder *sinnvollen* Wort angeboten wird: die selektive Auslösung der Erwartungswelle vor dem Li htreiz beweist die Differenzierung der beiden Wortgruppen mit Zuordnung eines Warncharakters für den dem Lichtreiz vorangehenden Wortreiz.

Bei ca. 20% der insgesamt 60 Versuchspersonen war trotz *normalem* Hörvermögen und Sprachverständnisses die Erwartungswelle nur sehr schwach ausgebildet und diagnostisch kaum zu verwerten. In diesem Zusammenhang wäre die Frage zu prüfen, ob in solchen zweifelhaften Fällen durch eine Steigerung der Zahl der angebotenen Testworte bessere Ergebnisse zu erzielen ind. Diesbezüglich Erfahrungen an einigen wenigen Fällen sprechen für diese Annahme.

Die Grösse der langsamen Hirnpotentialänderungen wird durch die Aufmerksamkeitszu oder Abwendung der Versuchsperson während des Ver uches beeinflusst. Rebert *et al.* (1967) haben darauf hingewiesen und auch wir konnten dieses Phänomen bei schwellennaher Stimulierung beobachten. Dabei muss die V rsuchsperson eine wesentlich grössere Konzentration aufbringen um die einzelnen Worte zu diskriminieren. Es ist daher verständlich, dass die Erwartungswelle grösser wird als bei überschwelliger Prüfung. Bei einigen schwellenn hen Untersuchungen konnten wir allerdings sowohl nach sinnv llen wie auch sinnlosen Worten Erwartungswellen nachweisen, selbst wenn nur einem der beiden Wortreize ein Lichtreiz nachfolgte. Wir fassen dies al Bewei dafür auf dass die Versuchsperson die beiden Wortgruppen nicht mehr mit Sicherheit unterscheiden konnte und daher auch inigen W rten jener Gruppe die keinen Warncharakter hatte einen solchen zu rdneten damit kam es auch beim Kontrollversuch zur Ausbildung ein r Erwartungswelle.

SUMMARY

In a ttempt to develop a method for objective determination of acoustic discrim n tion, umber of meaningful words and nonsensical acoustical equivalent were ffered t 70 person with normal audition. When the meaningful words wh were f llowed by a light flash, a slow negati potential shift (ex



Abb. 7 Vergleich der Erwartungswelle bei 75 dB (*Linke Kurvenpaar*) und 25 dB (*Rechtes Kurvenpaar*). Während bei überschwelliger Stimulierung nur nach sinnvollem Wort und Lichtblitz eine Erwartungswelle auftritt, findet man eine solche bei schwellennaher Stimulierung auch schon nach dem sinnlosen Wort allein.

Abb. 8 Erwartungswelle nach sinnvollem Wort und Lichtblitz unter Konzentration auf die Reizsituation (*oben*) und bei Konzentrationsabwendung (*unten*)

zunehmender Konzentration die Amplitude der Erwartungswelle zunimmt. Bei einigen Versuchspersonen ergab sich allerdings in Schwellennähe auch nach dem sinnlosen Wort, das als Vergleichsreiz allein angeboten wurde eine deutliche Erwartungswelle (Abb 7) Dies erscheint uns ein Hinweis darauf dass die Versuchsperson infolge der Schwellennähe des Reizes den Bedeutungsgehalt der beiden Worte nicht mehr mit Sicherheit differenzieren konnte

Bei einer Reihe von Versuchspersonen haben wir die Untersuchungen unter konzentrierter Aufmerksamkeit und während des Lesens eines Textes durchgeführt In den meisten Fällen wurde durch die Konzentrationsabwendung während des Lesens die Erwartungswelle deutlich verkleinert und mitunter auch völlig ausgelöscht (Abb 8)

DISKUSSION

Nach Anbietung sinnloser und sinnvoller Worte ohne nachfolgendem Lichtreiz ergeben sich gewisse Unterschiede in den gemittelten Potentialen wobei diese nach dem sinnvollen Wort meist ein etwas grössere Amplitude aufweisen Eine ähnliche Beobachtung haben Oswald *et al* (1960) an Hand der K Komplexe im EEG beschrieben Bei Kontrolle eines grösseren Versuchsmaterials zeigt sich jedoch dass diese Unterschiede kein verlässliches Kriterium für die gnostische Verarbeitung und Differenzierung zwischen den beiden Wortreizen darstellen

Verwertbar ist in dieser Hinsicht hingegen der Nachweis einer Erwartungswelle, wie dies in den drei Testprogrammen mit Unterscheidung zwi

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HISTOPATHOLOGICAL CHANGES IN THE AUDITORY PATHWAY IN CASES OF FATAL HEAD INJURY

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I five patients wh died 24 hours, 3 days, 8 days, a month, and 4 months, respecti ely after head trauma, the brain and the brain stem were histopathologically examined with special reference t the auditory pathway nd the cortex. In one case who survived for 3 days, hemorrhage in the brain stem affected the inferior colliculus, lateral lemniscus, and superior livary nucleus. In another case who survi ed for 4 months, multiple softening foci scattered in the brain stem affected also the inferior colliculus, lateral lemniscus, a d superior olivary n cleus. In remaining three cases, hemorrhage in varying degrees was bserved i the brain stem and the brain. From these fi dings it was assumed that i case f head trauma, hearing impairment may possibly be caused by retrolabyrinthine lesi n.

Although fractures of the skull d not always result from head injuries, varying kinds and degrees of damage occur in the middle ear sound transmis ion apparatus as well as in the inner ear Concomitant damage in the intracranial tissues, particularly of the central nervous system and blood vessels, may often occur and i well documented. However hitherto little attention ha been paid t the central auditory pathway and its pathological changes due to head injury

In five cases of fatal head blows of different survival periods, the auditory pathway wa tudied histopathologically in order to clarify the etiology of hearing lo s a sequelae of the head injury

C e Report with Pathology of the Brain and Brain Stem

In Table 1 a umm ry f five cases is made Age of the patients ranged from 24 to 56. F u cases among five had epi- or subdural h matoma They lost consciousnes and died 24 hours, 3 days, 8 days, a month, and 4 months, espectively after head trauma. Among these cases, two will be discussed in detail.

Staining Method of the Central Nervous System

After fixation in 10% form lin, sliced tissue of the brain and brain stem w s embedded in paraffin.

pectancy wave) was found in the time-linked EEG computer analysis prior to one light flash. This result indicates that the expectancy wave can be linked with the word contents, thus enabling an objective criteria of word discrimination.

RÉSUMÉ

L audiométrie par analyse de l EEG moyennant ordinateur permet d évaluer objectivement les seuils de stimulus sonores, mais ne permet pas de conclure à la compréhension de l excitation perçue. En comparant les réaction corticales à des stimulus par des mots chargés de sens ou non on constate des variations du tracé conformes à l information contenue dans l excitation On ne trouve une onde d attente q apres la perception des mots chargés de sens.

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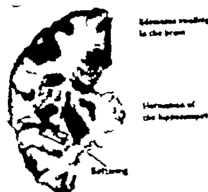
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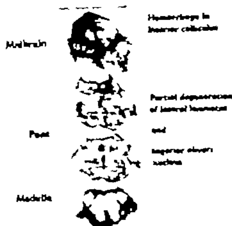


Fig. 1

Fig. 2.

Fig. 1 (case II) Left hemisphere. Marked edema i the hemi phere hippocampal hernia-tion ad soft g i th i ferior part of the temporal l be

Fig. 2 (case II) Brai stem. Hemorrhage in the midbrai nd the pons.

Superior olivary nucleus (Fig. 3) Normally stained ganglion cells and poorly stained ghost-like cells were seen. This partial degeneration was a sumed by a pathologist to be a retrograde degeneration secondary to hem rrhage in the inferior colliculus.

Lateral lemniscus There was necrotic lesion in the upper part of the pons in luding medial lemniscus. The *nucleus of the lateral lemniscus* did not show pathology

Inferi r colliculus Hemorrhage in the basal part of the midbrain affected the inferior colliculus.

Cyrus of Heschl (Fig 4) Virchow Robin s space around the ganglion cell were observed However it is hard to say whether this finding is patho-l gical

Case 3

A 32 year-old man was severely traumatized when he was struck by an utomobile A week later he lost consciousness accompanying by severe vomiting Con ulsion of the left leg occurred. A lumbar puncture showed evidence of increased pressure He was admitted to a hospital with a diag nosi f acute intracranial hemorrhage

On admis ion, he was in a state of coma. He responded only to pain sti-mul tion. There were fixed pupils, anisocoria, and chocked discs. Examina tion revealed exaggerated reflexes and Babinski s sign bilaterally X ray ex mination disclosed a fracture line of about 8 cm extending from the parietal region of the base of the skull. Angiography showed upward dis-placement of the left middle cerebral artery

A craniotomy resulted in removal of extradural hematoma of the right

TABLE 1 *Pathological changes in the central auditory system*

(—) No pathology — not examined.

No of cases	Age sex	Survival period	Cochl. nucl.	Sup oliv nucl.	Lat lem. nucl.	Inf collic.	Med. gen. body	Heschl gyrus
I	56, M	24 hrs	(—)	(—)	Hemorrh.	(—)	—	Hemorrh.
II	33 M	3 days	(—)	Degen.	Degen.	Hemorrh	—	Degen.?
III	24 M	8 days	(—)	Degen.	Degen.	(—)	—	Degen.
IV	39 M	1 mth	(—)	(—)	Hemorr and degen	—	(—)	Degen.?
V	32, M	4 mths	Softening foci degen	Softening foci, degen.	Softening foci, deg n.	Softening foci, degen	—	Softening foci, degen.

Sections were made in the usual manner Krüver Barrera's method was employed, which stained the myelin sheath blue, nuclei of the nerve cell and Nissl substance purple Azan staining was also employed

Case II

A 33-year-old man was struck by a falling log which fractured his skull He was brought to a hospital 10 minutes later Immediately after admission, he began complaining of parietal headache, vomiting, and losing consciousness Examination revealed anisocoria and fixed pupils Craniotomy was performed because extradural hematoma was suspected

Operation findings There was a marked elevation of intracranial pressure. Extradural hemorrhage covered the frontal parietal, and temporal regions of the left side After hematoma was evacuated, a fracture line was observed crossing the anterior branch of the middle meningeal artery near the parietal area, where bleeding still occurred. The temporal lobe and the parietal lobe were markedly compressed by hematoma Subdural hematoma was noted in the anterior cranial fossa as well as in the middle cranial fossa. Extradural and subdural hematomas weighed 200 g

The patient remained in coma and died 3 days after operation. The principal findings at autopsy consisted of extradural hematoma, hippocampal herniation of the left side fracture of the left temporal bone (Fig 1) There were edema and softening in the brain Hemorrhagic lesions were observed in the brain stem (Fig 2)

Microscopy of the central auditory pathways

Ventral cochlear nuclei, dorsal cochlear nuclei, and trapezoid body No alterations were seen.

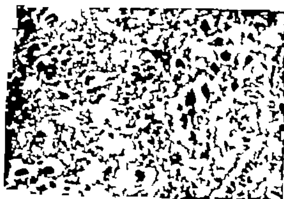


Fig 3.

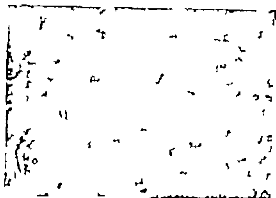


Fig. 4.

Fig 3 (case II) Superior olivary nucleus. Normally stained ganglion cells and poorly stained, atrophic ganglion cells.

Fig. 4 (case II) Heschl's gyrus. Appearance of Virchow Robin's space round the ganglion cells.



Fig 5

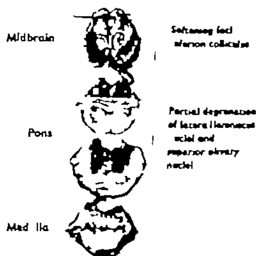


Fig 6.

Fig. 5 (case V) Cross-section of the brain. A softening focus in the inferior surface of the right temporal lobe

Fig. 6 (case V) Brain stem. Small softening foci in the midbrain and the pons.

temporal area (150 g). Following surgery coma persisted. The patient died of anoxia four months after operation.

Autopsy findings. Areas of encephalomalacia were seen in the lateral and inferior part of the right temporal lobe and in the inferior part of the left temporal lobe (Fig 5). Cross section of the brain showed marked subcortical dissemination of lesions of encephalomalacia. The brain stem showed smaller areas of encephalomalacia (Fig 6).

Microscopy of the central auditory pathways

Ventral cochlear nuclei. No hemorrhage. There was pathology due to softening.



Fig. 7 (case V) Lateral lemniscus nucleus. Some ganglion cells are atrophic and poorly stained. Gliosis and round cell infiltration are visible

Dorsal cochlear nuclei and trapezoid body No hemorrhage, no degeneration

Superior olivary nucleus No hemorrhage. The cytoplasm of the ganglion cells was poorly stained, suggesting retrograde degeneration.

Lateral lemniscus nucleus (Fig. 7) There was a necrotic lesion involving the nucleus of the lateral lemniscus. Ghost-like and atrophic cells were seen. Demyelinated area was observed. Gliosis and round cell infiltration were also seen.

Inferior colliculus Small softening area was observed

Gyrus of Heschl No alterations were seen

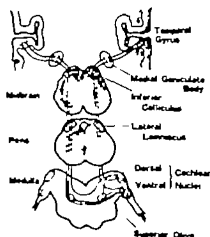


Fig. 8.

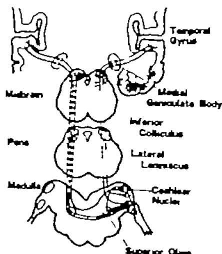


Fig. 9.

Fig. 8. Location of hemorrhage superimposed for cases I IV

Fig. 9. Softening foci (case V)

Case Analysis

By superimposing maps of hemorrhage of 4 cases, excluding the case which has the *softening foci* we can see the site of hemorrhage from the brain stem up to the temporal lobe (Fig 8) The map shows that hemorrhage occurs mainly in the midbrain and the pons, particularly in their dorsal part

Fig 9 is the schematic drawing of site of lesion in the brain and the brain stem in case V who survived 4 months. Small black areas indicate the location of softening foci in the midbrain, pons, and the temporal lobe.

Table 1 illustrates a review of localization of histopathological changes in five cases. It seems that the pathologic change, if present mainly extended from the superior olivary nucleus, through the lateral lemniscus, and up to the inferior colliculus.

DISCUSSION

Many papers have described histopathology of the brain after injury Pathology consisted of hemorrhage edema, degeneration, softening, and necrosis Pathology may differ in survival periods after injury Several kinds of changes may coexist in a case.

As to pontine hemorrhage, Attwater (1911) found 12 cases of head injury in 67 cases of pontine hemorrhage. Of these, four had fracture of the base of the skull and hemorrhage in the internal capsule. He stated that pontine hemorrhage was found in cases of severe damage of the parietal area, irrespective of damage of the base of the skull The pons was not an area of predisposition to hemorrhage The central area was rather more susceptible to hemorrhage than the periphery in the pons, even secondarily as a result of intracranial pressure elevation.

Wilson & Winkelman (1926) found 13 cases (10%) of pontine hemorrhage in 129 cases of autopsy Multiple hemorrhagic lesions were present in most of the cases. Extrapontine lesion was present in each case Wilson found five cases as the result of trauma in 13 cases of pontine hemorrhage although it was not clear when hemorrhage occurred The pons was indirectly damaged by impact which was transmitted from one hemisphere to the other

Moore & Stern (1938) postulated that an abrupt pressure change above and down the tentorium caused circulatory disturbance of the basilar artery then elevated arterial pressure and eventually led to hemorrhage in the pons. Dill & Isenhour (1939) thought that the pontine hemorrhage was *due to* compression of the pons into the foramen magnum Extradural hematoma was primarily responsible as it elevated the intracranial pressure

Beside the direct trauma to the brain stem, the impeding effect of the elevated intracranial pressure to *the venous drainage was thought to be* the main factor of the brain stem hemorrhage by Poppen *et al* (1952)

Arterial hemorrhage also occurred in the brain stem injury but only occasionally

Areas of predilection to venous hemorrhage in the brain stem was studied by Berner (1940) He investigated anatomy of the vein in the brain stem by intravenous injection of a lead substance, which was photographed by X ray There were large veins anterior to the brain stem. The vein in the floor of the fourth ventricle was less vascularized. These findings proposed a problem against the concept that the fourth ventricle was an area of predilection to the hemorrhage On the other hand, venous hemorrhage was found in the patient who survived more than 48 hours.

Nakamura (1967) found hemorrhagic lesion in the brain stem of a monkey which died instantly by an experimental falling down. The intracranial pressure was measured and proved to be biphasic, negative, and positive.

He stated that the lesions in the brain stem were produced in the following way an impact distorted the brain within the skull, then the stress caused by distortion concentrated to the brain stem resulting in injury

The present cases survived and remained unconscious several hours, 3 days, a month, and 4 months, respectively after the injury This indicated the lesion was extremely severe. Four cases out of five showed extra- and/or subdural hematoma. Besides affection of the cerebrum by pressure of the extradural hematoma, edema, softening (cases II and V) subcortical hemorrhage of the right temporal lobe (case I) and Virchow Robin s space formation around the ganglion cells in the gyrus of Heschl (cases IV and II) were also observed.

Hemorrhage in the brain stem was considered to be produced by compression of the subdural or extradural hematoma (Dill & Isenhour 1939) or secondarily by herniation of the medial part of the temporal lobe or of the hippocampal gyrus which compressed and distored the brain stem (Nelson, 1942 Courville, 1945) As a matter of fact, hippocampal herniation was observed in case II. It was hard to decide when hemorrhage occurred in the cases which did not show herniation. In the brain stem, hemorrhage was located in the upper pons (case IV) the inferior colliculus (case II) and the medulla near the outer portion of the posterior area (case I)

The lesion in olved the auditory pathway producing degeneration of the lateral lemniscu and its nuclei (cases IV II, and I) and the inferior colliculu (case II)

Case V is instructive when we consider the pathology of sequelae of head injury The patient lived 4 months after injury the longest survival in this series of study Histology showed small areas of encephalomalacia in the temporal lobes. In the brain stem, cochlear nuclei, the superior olivary nucleus, the lateral lemniscus nucleus, and the inferior colliculus were degenerated by disseminated lesions of softening.

The present cases showed that hemorrhage and degeneration occurred either in central or peripheral area f the brain stem in the cross-sections, although the latter was rather common.

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As already described previous papers paid consideration on areas of pre dilection of brain stem lesions In our cases, blood vessel distribution in the brain stem was closely related to the location of the lesion. Hemorrhage in the peripheral area accompanied hemorrhagic necrosis in the lateral lemniscus and inferior colliculus.

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RÉSUMÉ

Une étude histopathologique du système nerveux central est faite sur quatre autopsies de blessure fatale de la tête, spécialement à l'égard du cortex et des voies auditives. Il se trouve qu'il y a des régions d'hémorragie et de ramolissement de divers degrés lelong des voies auditives, spécialement dans le nucleus olivaire supérieure le colliculus inférieur et le lemniscus lateral. Sur le fondement de l'étude présente une discussion est faite sur la caractéristique de la diminution auditive secondaire à une blessure de la tête.

ZUSAMMENFASSUNG

Das zentrale Nervensystem wurde in 5 Autopsien von Fällen mit tödlichen Schädelverletzungen histopathologisch untersucht unter besonderer Beachtung der Hörrinde und der akustischen Bahnen Man fand, dass stellenweise Blutungen und verstreute Erweichungen in unterschiedlichen Stadien entlang die akustischen Bahnen besonders in der oberen Olive, der lateralen Schleife, dem unteren Vier hügel vorhanden waren Auf Grund der vorliegenden histologischen Untersuchungen hat sich ein Anhalt dafür ergeben dass wenige Fälle mit dem Kopf trauma die Schädigungen auf die akustischen Bahnen haben und dadurch die zentralen Hörstörung zeigen

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DISCUSSION

J. J. Groen: I have two questions. (1) With what method has he determined the interaural time difference? (2) I have the impression that Mr Kirikae puts the borderline between normal and abnormal interaural time difference at 60 micro-seconds. We have the experience that none-too-careful, but otherwise normal, listeners need sometimes 90-100 microseconds. We would call a pathological case one who needs 200 or more microseconds interaural time difference. Would Mr Kirikae agree upon this with us?

J. McA. Uffe Curtin: This very interesting paper deals with a problem which is increasing, namely deafness following head injuries due to automobile accidents. I would like to ask Mr Kirikae if all the fatal cases had a transverse or longitudinal fracture of the skull? Secondly, if any of these patients exhibited a blue tympanum on many occasions. The deafness is often unnoticed by the patient because of the severity of the cerebral injury. Lastly, has he observed many cases of spontaneous recovery when the deafness appeared early after accident?

I. Kirikae (Reply): 1. Mr Groen. To measure the noticeable time difference we use the earphone method. By changing the distance between two microphones situated in the interaural time difference adjusting apparatus, we can get variable time difference of test tones entering into both ears independently of the intensity difference. Test tone was interrupted band noise (2 times/sec) *of 600–800 Hz.* We estimated the value of 0.06 msec as a standard, and when the test result is less than this value, we evaluated it as normal. But, when the test result exceeded this value we suspected the *existence* of retrocochlear lesion.

To Mr *M. A. Uffe Curtin*. (1) I have several cases of hearing impairment following head trauma who recovered spontaneously. I think it would be possible to recover because in case of unconsciousness it can become conscious if the brain lesion is not organic but rather functional one. When hearing impairment is caused by functional disorders of the auditory organ and the brain itself, it will be possible to recover. However if the lesion in the auditory system is organic, i.e. hemorrhage in the brainstem, even though it is small, hearing impairment will not recover. On the contrary I know a case whose hearing impairment gradually aggravated for 4 months following head trauma. (2) Fractures of the skull were found in three cases out of five autopsied cases. From the observation of surviving patients who have hearing impairment following head trauma, no close relation was found between skull fracture and occurrence of retrolabyrinthine deafness. Fracture of the skull has rather connection with unilateral inner ear deafness. (3) Concerning the incidence of blue tympanum in our cases, I have no precise data at the present time.

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THE COCHLEA AND THE COCHLEAR NUCLEI IN THE BAT

Plecotus auritus

J G HALL

From the Anatomical Institution and Rikshospitalet E N T Department University of Oslo Oslo Norway

Twenty specimens of *Plecotus auritus* the most common bat in Norway were collected from an old church tower Ten animals were used for the preparation of the cochlea the others for the preparation of the brain and the 8th nerve. For the preparation of the cochlea the osmium tetroxide fixation and the surface preparation technique described by H Engström *et al* (1966) was applied.

The brains were fixed in 10% formaline, sectioned in 10 microns and stained with thionine.

The method described by the author (1964) was applied for computing the volume of the cochlear nuclei and for counting their number of nerve cells. Also some data concerning the outer and the middle ear of these animals were collected.

Measurements of the outer ears, the ear-drum, the cochlea, and the basilar membrane were reported. Pictures from these parts of the animals were shown the organ of Corti and the form of the hair cells was discussed. A counting under the microscope showed that 50 of the inner hair cells occupied the same space as 60 in one of the outer rows. It was also shown that the narrow space occupied by the basilar membrane was due to the development of a lamina spiralis ossea secundaria.

The cochlear nuclei were then discussed, their spindle-like form was shown, and their cubic content calculated. Their main sections, the anteroventral, the posteroventral, and the dorsal nucleus were shown in serial square sections. The number of nerve cells of the cochlear nuclei in these bats, 40 000 was compared to the number of cells in the cochlear nuclei of other species employing echo-location especially the whales, and also to the number of nerve cells in the cochlear nuclei of man and cat The role of the dorsal cochlear nucleus in these species was especially discussed. The distribution of the nerve fibres within the nuclei, shown in a picture prepared by a nerve-fibre staining was compared to the frequency distribution within the nuclei

RÉSUMÉ

L oreille externe et interne chez la chauve-souris est décrite ainsi que les noyaux cochleaires. L Auteur a collectionné 20 exemplaires provenant d une vieille tour d église en Norvège. Dix exemplaires ont été utilisés pour preparer la cochlée d après la méthode de H Engström le reste a été utilisé pour preparer les noyaux cochléaires dans le bulbe, d après la méthode que l auteur a décrit en 1964

ZUSAMMENFASSUNG

Das äussere und innere Ohr der Fledermause, Plecotus auritus, wird beschrieben, sowie die Cochleariskerne derselben. Der Verfasser hat 20 Exemplare in einem alten Kirchturm i Norwegen gesammelt. Von 10 Exemplaren sind die Cochleae nach der Methode von H Engström präpariert, n den anderen 10 sind die Medullae nach der von dem Verfasser in 1964 beschriebenen Methode präpariert worden.

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The Anatomical Institution and Rikshospi talet E.N.T Dept University f Oslo, Osl Norway

DISCUSSION

G d Wit In whales there i intensi e intercon ection between the right d the left cochlear uclei, even so intensive that it is lready microscopically isible I this the same case in bats?

J G Hall (Reply) to Mr *d Wit* It is true that the corpus trapezoidum is gre t also i the li y bats, but the fibres d not cross from th cochlea u lei on the on side to the other but tra erse a d course t the lateral lemniscus on the other side

Owing t the umber of illustrations belonging t thi work, the article could not be included i this issue but will be published, in its full ersion, in mber 3 f thi ol me

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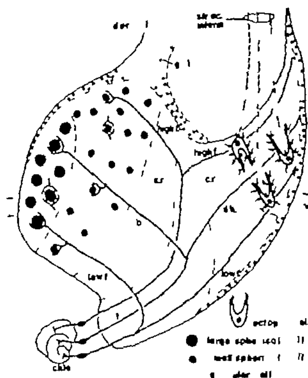


Fig. 1 Diagram of the t entral cochlear ucleus i the sagittal plane The bifurcatio nd tonotopical organization of the cochlear ner fibers (f) are shown. Except for the superficial layer of gra lar cell (g.c.l.) only the distribution nd type of 1 nerv tion of the thre cell types under considerati are indic ted. For further expl nation, see the t t. b Ascending cochlear branches; c central region d.b descendi g cochlear branches; high f high tonal frequencies; low f low tonal frequencies; i ac. int rm., intermediate acoustic stria.

cochlea projects ventrally and the basal part dorsally (Fig. 1) Accordingly in the cochlear nuclei high t nal frequ ncies are represented dorsally or above and low frequencies ventrally or below The course of the ascending and descending cochlear branches differs fundamentally the ascending branches tend t diverge while the descending ones are distinctly conver gent

The cells in the anterior part f the ventral cochlear nucleus appear round without visible dendrites in Nissl sections, and I have therefore called them pherical cells. According to their size I have classified them as two types, viz., *large and small pherical cells* In Nissl preparations these two cell types ppear much alike they are both characterized by a peculiar *arrange* ment of the Ni sl substance which forms a distinct nuclear cap and a concentric ring of coarse granules (Fig. 2 I *B*) Both types are supplied from the scending cochlear branches by means of typical bulbs of Held (Fig. 3 I *B*) which represent a special type f synaptic endings (Held,

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THE INTRINSIC ORGANIZATION OF THE COCHLEAR NUCLEI IN THE CAT

KIRSTEN KJLLSBERG OSEN

From the Anatomical Institute University of Oslo Oslo Norway

In the cochlear nuclei nine different cell types are distinguished on the basis of Nissl preparations. Only three of these cell types are presented here *The large and small spherical cells* are innervated from the ascending cochlear branches by means of typical bulbs of Held. The former cell group is apparently supplied only from the apical and middle part of the cochlea and projects bilaterally on the medial superior olive, while the latter group seems to be supplied from the entire cochlea and projects on the lateral superior olive on the same side. *The octopus cells* are innervated from the descending cochlear branches by means of small ring shaped boutons. The axons of these cells run in the intermediate acoustic stria and terminate probably in the retroolivary and medial preolivary nuclei on both sides. The function of the three types of cells is discussed.

The cochlear nuclei form the first relay center in the ascending auditory pathway In these nuclei each cochlear nerve fiber establishes contact with several different types of cells, most of which project further centrally Thus the impulses which are set up in the cochlea become transferred into different paths leading up to higher auditory centers. Although these paths may be integrated at all levels, the organization of the cochlear nuclei presumably provides a key to a better understanding of the entire auditory system

On the basis of Nissl preparations nine different cell types may be distinguished in the cochlear nuclei of the cat and I have proposed a parcellation of the entire complex based exclusively upon the distribution of these cells. A satisfactory description of all cell types would be impossible in a quarter of an hour and therefore only three of them will be presented here

In mammals the cochlear nuclei consist of a ventral and dorsal nucleus. The cells in question viz., the large and small spherical cells and the octopus cells, occupy the most anterior and posterior parts of the ventral nucleus as shown in Fig 1 The central region of this nucleus contains a mixture of cell types which are not being commented upon here

According to the Golgi studies of Cajal (1909) and Lorente de Nó (1933) all cochlear nerve fibers divide into ascending and descending branches. As shown anatomically by Sando (1965) and physiologically by Rose *et al* (1959) the branches are arranged tonotopically so that the apical part of the

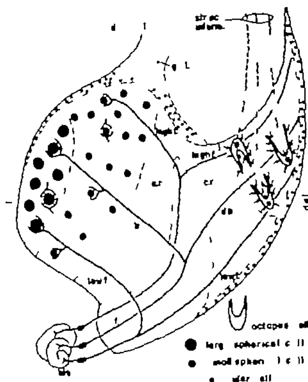


Fig. 1 Diagram of the ventral cochlear nucleus in the sagittal plane The bifurcation and tonotopical organization of the cochlear nerve fibers (f) are shown. Except for the superficial layer of granular cells (g.c.l.) only the distribution and type of innervation of the three cell types are considered. For further explanation, see the text. *a.b.* Ascending cochlear branches; *c.r.* central region; *d.b.* descending cochlear branches; *high f.* high tonal frequencies; *low f.* low tonal frequencies. *st. ac. interm.* intermediate acoustic stria.

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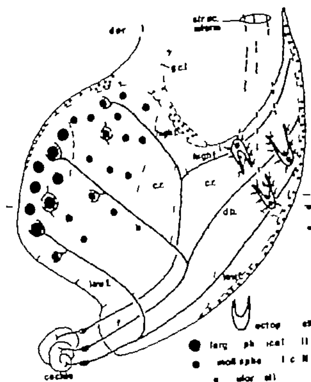


FIG. 1 D gram of the cat entral cochlear nucleus I the sagittal plane. The bifurcation and tonotopical orga ization of the cochlea nerv fibers (*c.f.*) are shown. Except for the superfic l layer of gran lar cells (*g.c.l.*) only the distribution nd type of innerv tion of the three cell types unde cons deration are indicated. For farther explanation, see the text. *b* Ascendi g cochlear branches *c.r.* central regio *d.b.* descending cochlear branches; *high f.* high tonal freq encies *low f.* low tonal frequencies

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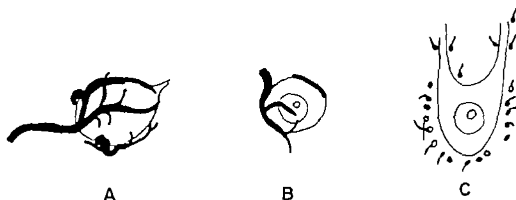


FIG 2. Ph tomicrographs of large spherical cells (*A*) small spherical cell (*B*) and an octopus ll (*C*) 15 μ section Nissl stain 480

1893 Cajal 1909) The cell bodies are usually embraced by two or three bulbs which probably means that only a few cochlear nerve fibers converge upon each cell Also the large number of these cells, as compared with the other cell types of the cochlear nuclei, is compatible with a slight degree of convergence of primary afferents in this region

As shown in Fig 1 the large spherical cells are densely packed in a cap-shaped area which does not reach the dorsalmost part of the nucleus. These cells, therefore, are probably concerned merely with the conduction of low and middle tonal frequencies The small spherical cells, on the other hand, form a broad band of cells which crosses all the ascending branches from high to low and thus may be engaged in the conduction of all frequencies.

Despite their similar appearance the large and small spherical cells definitely have different central connections. I have studied the course of their axons by means of anterograde degeneration following restricted lesions of the cochlear nuclei The lesions were made stereotactically by electrocoagulation, and the sections were stained by the silver methods of Glees



Fi 3. Drawing f bulb of ll ld upplying l rg ph rical cell (A) nd small pheric l cell (*B*) nd ri g haped boutons upplyi g an oct pu ll (*C*) 15 μ section stained by th m thod f Glees

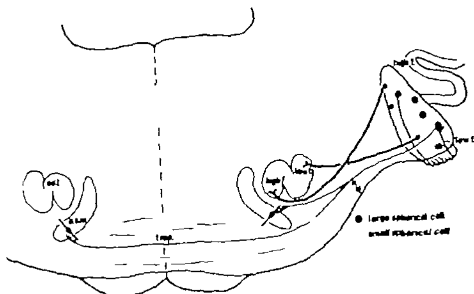


Fig. 4. Diagram of the medulla in the transverse plane showing the location and projection of the large and small pherical cells. The di lslo f the larg cell ax s, indicated here, has not been proved. F further planation, see the t xt. ca. *trap.* Trapezoid body; *high f* high tonal freq ncies *low f* low tonal frequencies *s.l* lateral superior oli ; *s.m* medial superior oli e.

and Nauta. This technique makes it possible to follow the degenerated nerve fibers from the place f lesion to their *site of termination* In all 30 cats were used in these experiments and from a comparison of a great number of cases it appeared that the large spherical cells project bilaterally upon the medial superior olive while the small ones project on the *lateral superior* olive on the same side (Fig 4) Only the main projection of the cells is con idered here while possible additi nal projections to other nuclei are left ut of account

The cells f the medial superi r olive are bipolar and arranged in the transverse plane. Each cell is innervated bilaterally from the cochlear nuclei the right dendrites from the right side and the left dendrites from the left side Stotler showed this in 1053 but he did not recognize from which part of the cochlear nuclei these fibers originated. As each cell of the medial superior olive receives impulses from both ears, physiologists have suggested that this nucleus is concerned in the localization of sound. More particularly the medial superior olive may be invol ed in the sound-eye reflexes as it, according to the comparative anatomical study of Irving & Harrison (1967) is especially well developed in animals with large eyes. On the ther hand, it is definitely not involved in the echolocation mechanism of the porpoise and bat as in these animals both the medial superior olive and the large spherical cells are rudimentary The fact that these animals are mainly adapted for ultrasonics supports my *suggestion that*



FIG. 5 Photomicrograph of the octopu cell area (oca) with the adj cent parts of the ntral gion f th ventral chl ar nucleus (c) and the granular ll l yer (gcl) The ertical lin ndicates the bord r between the oca nd the c The dendrites of the octopu cell are o iented v rtically across the direction of th unst ined descending cochlear branches, the direction of which is indicated by rrow 50 μ sagittal section tained f r cetylcholi esterase according to a modificati n of the thiocholin method of Koelle & Friedenw ld. Incubati n with ac tylthioch lin f r 2 h urs. I hibitor Mipa f x × 110

this portion of the auditory system is operated mainly by low tonal frequencies.

The small spherical cells project on the lateral superior olive in a strict tonotopical manner the dorsal part of the area projects medially and the ventral part laterally as shown in Fig 4 Accordingly the lateral superior olive should be tonotopically organized so that low tonal frequencies are represented laterally and high frequencies medially and that is exactly what was found physiologically by Tsuchitani & Boudreau (1966) in the cat According to their findings, moreover all tonal frequencies audible to a cat are represented in the lateral superior olive, an observation which is highly compatible with my suggestion that the small spherical cells are supplied from the entire cochlea The strict tonotopical organization the low degree of convergence, and the relation to all frequencies apparently make this portion of the auditory system especially well suited for frequency analysis. Interestingly both the area of small spherical cells and the lateral superior olive are remarkably large in the porpoise which is capable of hearing tones as high as 150 kc/s or more

Fig. 4. Diagram of the medulla in the transverse plane showing the location and central projection of the octopus cells. The retroolivary and medial preolivary nuclei are indicated as black cells. For further explanation, see the text. *co. rf.*, Restiform body; *co. tr. p.*, trapezoid body; *co. trap.*, trapezoid nucleus; *nc. coch. dors.*, dorsal cochlear nucleus; *c.b.*, olivocochlear bundle; *o.s.l.*, lateral superior olive; *o.s.m.*, medial superior olive; *str. ac. dors.*, dorsal acoustic stria; *str. ac. int. rm.*, intermediate acoustic stria; *tr. sp. V*, spinal fifth tract.

The octopus cells are situated in the caudalmost part of the ventral cochlear nucleus (Fig. 1). In contrast to the spherical cells they are characterized by their thick, straight dendrites which course vertically across the direction of the descending cochlear branches. Due to the arrangement of their dendrites these cells often look like octopuses and therefore I proposed that name for them. In Nissl sections their perikarya show very fine Nissl granules and only the roots of the dendrites are visible (Fig. 2 C). The dendritic pattern becomes more conspicuous in sections stained for acetylcholinesterase according to the Koelle method, as seen in Fig. 5. The same cells have also been described by Harrison & Irving (1966) who classified them as k-cells. As the octopus cells are situated at the site where the descending cochlear branches are most closely packed the dendrites of each cell evidently cross a great number of branches. Both the perikarya and the dendrites are innervated from these branches by means of small ring-shaped boutons (Fig. 3 C). Though it remains to be definitely proved, the anatomical features suggest that each octopus cell, in sharp contrast to the spherical cells, is innervated from a great number of cochlear nerve fibers. The small number of octopus cells also favour the assumption that a considerable convergence of primary afferents takes place upon them.

Although the central projection of the octopus cells is not defined in all details, there exists some evidence for the main course of their axons. From



Fig. 5. Photomicrogr ph of the oct pus cell area (*oca*) with the djace t parts of the ce tral regi n of the entral ochlear nucleus (*cr*) and the granul r cell l yer (*gcl*) The ertical lin indicates the border between the oca and th cr The dendrites of the oct pu cells re ori nted rtically across the direction of th unstained descending cochlea branches, th di ection f which is indicated by arrow 50 μ sagittal section tained fo a ctylcholine t rase according to a modific tion of the thiocholine method of Koell & F iedenwald. Incubati n with acetylthiocholine for 3 hours. Inhibitor Mipa f x 110

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my own experimental studies in kittens it appeared that the octopus cells project centrally via the intermediate acoustic stria (Fig 6) a few days after the stria was cut the octopus cells showed definite signs of acute retrograde degeneration. In these experiments the stria was cut just medial to the restiform body and the course of the axons could not be determined beyond this point However the central course of the intermediate acoustic stria was recently shown by Fernández & Karapas (1967) in experimental anatomical studies on adult cats. They found that the fibers terminate bilaterally in the retroolivary and medial preolivary nuclei (Fig 6) As these nuclei give origin to the olivocochlear bundle of Rasmussen (1946) one may suggest a relation between the octopus cells and the bundle. Though it is not definitely proved, it seems reasonable that there exists here a short reflex arch composed of three links, namely the spiral ganglion cells, the octopus cells, and the cells of the retroolivary and medial preolivary nuclei. The presumed convergence of auditory impulses upon the octopus cells apparently makes them particularly well suited to form a link in this inhibitory feed back mechanism

RÉSUMÉ

Une division des noyaux cochléaires est proposée Elle est fondée sur la séparation microscopique de neuf groupes cellulaires différents. Parmi ceux-ci, trois groupes sont décrits au point de vue de leur rôle possible dans l audition

ZUSAMMENFASSUNG

In den Cochlearis-kernen der Katze lassen sich in Nissl-Präparaten neun verschiedene Zelltypen unterscheiden von denen drei hier besprochen werden Aufsteigende Cochlearisäste innervieren *die grossen und kleinen sphärischen Zellen* unter Bildung der typischen Held schen kolbenartigen Anschwellungen Die grossen Zellen werden offenbar nur vom apikalen und mittleren Teil der Cochlea versorgt und sie projizieren bilateral auf die mediale obere Olive. Die kleinen Zellen scheinen von der ganzen Cochlea versorgt zu werden und projizieren auf die laterale obere Olive der gleichen Seite *Die Octopus ellen* werden von absteigenden Cochlearisästen unter Bildung von kleinen, ringförmigen Boutons innerviert. Die Axone der Octopuszellen verlaufen in der Stria acustica intermedia und enden wahrscheinlich bilateral in retroolivaren und medialen präolivaren Kernen. Die Funktion der drei besprochenen Zelltypen wird diskutiert

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Fig. 1. ll phasic est bula imbalanc fter the introductio f NaCl-sat rated solution in the middle the left ide 1st phase "irrit ti gns.

Fig. 2. Bipha ic imbalance after the introduction of NaCl-s t rated sol tion in the middle ea on the left side 2nd phase pa lytic signs.

ion and molecules. Thi exchange ought to be regul ted also by the osmotic or hydrodynamic pr sure

The introduction f a saturated solution of NaCl (0.2 ml) into the middle r f guinea pig caused an acute biphasic vestibular imbalance the experimental animals showed, a few minutes after the injection, a vestibular picture f the "irritative type of nystagmus in r lation to the injected side, nd t nic deviation of the head, trunk, and limbs towards the other side.

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MODIFICATIONS OF THE OSMOTIC PRESSURE OF PERILYMPH AND ENDOLYMPH

An Hypothesis on the Pathogenesis of Menière s disease[1]

M ARSLAN

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The introduction of a saturated solution of NaCl into the middle ear of a cat or guinea pig provoked a biphasic vestibular spontaneous picture which is composed of a 1st phase of "irritative" signs (nystagmus beating towards the injected side etc.) and of a 2nd phase of "paralytic" signs (nystagmus beating towards the non injected side, etc) Histological, electrophysiological, biochemical, and biophysical (osmotic pressure measurements) investigations have been carried out in many series of experiments performed by the author and his collaborators. It has been demonstrated that the changes of osmotic pressure between the perilymph and the endolymph are the essential pathogenetic factors of all clinical histological, and electrophysiological manifestations occurring in the experiment. Close analogies are indicated between the effects of the experiment and the pathogenetic factors and evolution of Menière s acute attacks in fact a Menière s attack shows "irritative signs followed by "paralytic signs. Furthermore, in these experiments and in a Menière s attack there are identical histological pictures of a "collapse" of the endolymphatic duct

It is generally admitted that pathologic modifications of secretory and biochemical mechanisms regulating the perilymphatic and endolymphatic circulation are the basic pathogenetic factors of labyrinth hydrops (Menière s disease) The continuous and precise processes of the reciprocate regulation of the perilymphatic and endolymphatic circulation depend (1) on the secretion and absorption mechanisms of the two liquids, and (2) on the permeability of the labyrinth membranes and structures. As many authors have demonstrated (Altmann & Waltner 1950 Rauch, 1966 Tonndorf 1957 etc.) exchange of water ions, and other substances between perilymph and endolymph appears highly possible, as their chemical characteristics are completely different In fact the separating membranes, such as Reissner s membrane and other membranes, may be passed through by water

[1] Thi pape i ynthesis f a larg numbe f experim ntal studies of diff rent branches (hist l gic l bioch mic l, bi physical, etc.) The in estigatio s were carried out mainly by Dr G M linari, M.D with th collaboration f D O Sala, M.D Dr F Giacom lli, M D G P rin, Ph.D and D W Mozzo, M.D all f whom re members of the E.N T Dept. f the Uni rsity f Padua.

phatic duct and modifies the functional conditions of the receptors (vestibular "irritative" signs).

5. Compensation mechanisms in the secretory structures of the stria vascularis and perilymphatic walls (such as a rapid increase of the ultrafiltration of water) overturn the vestibular imbalance (vestibular "paralytic" signs) and, finally, reestablish in about 1–2 hours the osmotic balance and, consequently, *the functional state of the receptors*, which return to normality. Fig. 3 is a schematic drawing of this hypothesis.

In order to prove in an objective way the validity of these hypothesis, we first had to demonstrate the semipermeability of the round window membrane, that is, the passage through it of the liquid (perilymph) having lesser osmotic pressure to the liquid of the middle ear having greater osmotic pressure (*hypertonic solution*).

Various-sized NaCl crystals (mol. weight 58.46, high constancy of electrolytical dissociation, stable neutral pH) were applied on the round window of anesthetized cats (sodium pentobarbital, 50 mg/kg intraperitoneally) while in a series of control animals crystals of methylene blue were employed.

After the Deposition of an NaCl Crystal on the Round Window Membrane (Fig. 4) we could observe

1. Appearance of perilymph on the membrane in 15 to 18 seconds.
2. Dissolution of crystal and progressive reduction in size.
3. Complete filling of the fossa of the round window with perilymph in nearly 3 minutes, so that the crystal was no longer visible.

Note added in proof. Following a suggestion of H. Spoendlin, the histological effects of the apposition of a NaCl crystal upon the round window was studied by G. Babighian of our department. The round window bilateral membranes of cats were exposed via the retroauricular approach and the opening of the bullae. On the membrane of one side a sodium chloride crystal was deposited, the contralateral membrane served as a control. Crystal dissolution caused water to leave the perilymph; water was removed after complete dissolution of the NaCl crystal (4 min.) and the round window membrane was embedded in paraffin and stained with haematoxylin-eosin. We observed the following histological modifications (Fig. 5).

The low cubic epithelium lining the round window tympanic wall appeared coarctated and disarranged, so that its morphological details were hardly identifiable. By some preparations, cells appeared detached from the basement membrane.

The fibrous connective layer which supports the round window membrane appeared markedly oedematous, the interstitial fluid was collected in small spaces and slits of the tissue enlarging it. Moreover, some slits were partly filled with a homogeneous, lightly acidophil material. We never observed phlogistic cells nor nuclear damage of the fibrocytes. The mesothelial cells were swelled out, but there was no other pathological aspect. Fig. 6 shows the contralateral control round window membrane (normal).

We can state that the passage of perilymph water (and perhaps of ions, too) from the scala tympani to the middle ear through a semipermeable mechanism, occurring in the round window membrane, is made possible by structural lesions induced from hypertonic solution formed after NaCl crystal apposition.

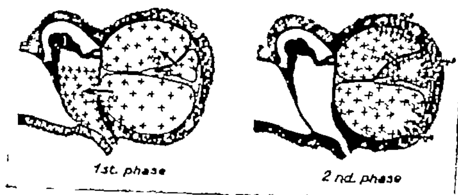


FIG. 3. Schematic representation of the experiment *1st phase* Water passes from the perilymphatic space to the middle ear owing to the diff rent osmotic pressures. From the endolymph, too, there is *probably* a passage of water to the perilymph and consequently the endolymphatic canal is compressed and collapsed. *2nd phase* After osmotic equilibration between th middle ear solution and the perilymph, water loss of the perilymph is quickly compensated by the ultrafiltration mechanism, and so osmotic difference arises between perilymph and endolymph, which is now hypert nic. Consequ ntly water pa ses from th perilymph t the nd lymph, so that th endolymphatic canal bulges.

These phenomena lasted from 15 to 30 min In the second phase of the experiments, beginning 40–50 min after the injection, the same vestibular signs reappeared but in the opposite direction ("paralytic" type) lasting from 60 to 90 min (Figs. 1 and 2)

Nearly 2 hours after the injection, animals no longer presented signs of labyrinthine disfunction and they returned to a perfect normality as a matter of fact thermic stimulation of the labyrinth of the injected ear at this moment gave values of provoked nystagmus equal to those obtained before the experiment (normal reflectivity) (Arslan, 1934)

The hypothesis formulated by us for the interpretation of the mechanisms of this experimental finding was as follows

1 The introduction of hypertonic saline solution into the middle ear produces in this cavity an high osmotic pressure As is known, hypertonic or saturated solutions injected into mucous-lined cavities are slowly diluted by water drawn from the interstices of the mucous membrane and its cells and vessels.

2 High osmotic pressure in the middle ear could provoke a passage of perilymph through the round window from the perilymphatic spaces to the middle ear

3 This passage of water is made possible because the round window membrane became a semipermeable membrane

4 The loss of water inside the perilymph provokes an acute crisis in the osmotic balance between the perilymph and the endolymph which probably causes a passage of water from the endolymph to perilymph through Reissner's membrane This fact is responsible for the collapse of the endolym





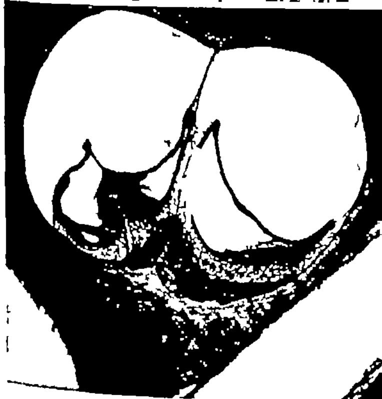


FIG. 5 Round window membrane (cat) after ppeition of \ Cl crystal for 5 minutes.

FIG. 8 Normal round w ndow membrane (cat) (control

FIG. 9 Collapsed ndolymphatic duct (1st phase of the perimenti "irritati estibular signs The cupola ppears compressed on the undamaged crista.



F a 4 Apposition f an N Cl cryst l n the round window ni he (On the ight side—up —the stapes and th l wi d w)

Figs. 5 a d 6 See col ur plate between pp. 364–5

Fig 7 The N Cl cry tal i compl t ly dissol ed perilymph is erfl wing from the round window niche to the promontorium and th middl ear

4 Overflow of the perilymph from the round window fossa in to the bulla and the subtympanic cavity of the middle ear after 4 to 5 minutes (Fig 7)

5 Stopping of the perilymph overflow

As to the Deposition of a Crystal of Methylene Blue we observed

1 Appearance on the window of a dark blue liquid after nearly 1 minute, that is, the outflowing perilymph set free in the methylene blue which is so-coloured.

2 Disappearance of the crystal in 7 to 8 minutes.



Fig. 5. Round window membrane (cat) fter pposition of N Cl crystal f 6 minutes.

Fig. 6. Normal round window membrane (cat) (control)

Fig. 9 Collapsed ndolymphatic duct (1st phase f the experiment. rritativ estibular signs) The pula ppears compressed on the undamaged crista.

3. Overflow of the dark blue liquid from the round window fossa after 18 to 19 minutes.

4. Stopping of the perilymph overflow

In order to verify the structural modifications, the electrophysiological phenomena, and the biochemical and osmotic changes which, in our experiment, have been provoked after the loss of water of the perilymph through the round window membrane to the middle ear cavity we carried out a series of different investigations.

A. *Histological Modifications*

The labyrinths of animals sacrificed at different times after the introduction of a saturated saline solution into the middle ear were studied on sections prepared after the modified Wittmaack's technique, both in the injected and in the non injected side (control series)

1 *During the first irritative" vestibular phase we observed*

Important modifications at the level of the cochlear canal of the injected side the findings were very similar to histological pictures found at post mortem examinations of the labyrinthes of Menière's patients, as described by many AA.

The perilymphatic space appeared extremely dilated and the endolymphatic canal was compressed and collapsed the tunnel of the organ of Corti remained visible even if the tectorial membrane adhered to the hair cells. In some preparations an homogenization of the protoplasm, both of hair cells and supporting cells, has been observed, so that in some cases it resulted difficult the identification of their cellular contours. The nuclei, however appeared unaffected (Fig. 8)

The same alterations appeared in the vestibular apparatus (Fig 9)

2. *During the second paralytic" vestibular phase we observed*

The cochlear canal appeared dilated and, while the tunnel of the organ of Corti appeared everywhere compressed, the hair cells and supporting cells showed only modest lesions (Fig 10)

B. *Cochlear Microphonic Variations*

In other series of cats we carried out investigations on the modifications of cochlear microphonics occurring during the different times of the experiment.

The recording electrodes of enameled silver wire, 150 microns in diameter were insulated up to the round window membrane and fixed to the edge of the bulla with dental cement.

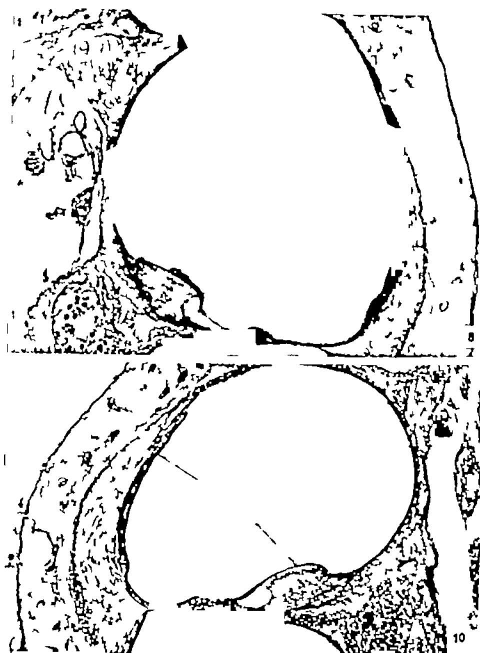


Fig. 8. Collapsed cochlear duct and dilatation of the perilymphatic space (the organ of Corti appears undamaged) (1st phase of the experiment).

Fig. 9. See colour plate between pp. 364–5.

Fig. 10. Bulging of the endolymphatic duct: the tectorial membrane is compressing the undamaged organ of Corti.

The indifferent electrode of silver wire was fitted in the muscles of the neck. Sound stimuli of 250, 1000, 2000, 4000 and 8000 cps (Peters audiometer mod. SPD 5) were given.

We fixed a speculum in the external auditory meatus of the animal and connected it to a loudspeaker through a rubber tube of 100 cm length.

The cochlear microphonic (C.M.) potentials from the round window were

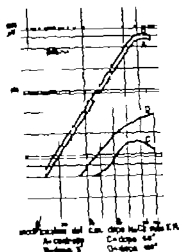


Fig. 11. Modifications of the cochlear microphonics after apposition of an NaCl crystal on the round window membrane (controll = control, dopo 3' = after 3', dopo 60' 120' = after 60' 120').

amplified with an A.C. preamplifier (Tektronix, mod. 122) and were recorded with an oscillograph (E.T.C., mod. h 21 A). The C.M. were recorded before having put a small crystal on the round window membrane and after having observed the outflow of the perilymph.

We can summarize our observations as follows:

1. Increase in C.M. immediately after the placement of a crystal of NaCl on the round window. This process ended after 1 to 2 minutes, in which time the crystal was completely diluted.

2. After the first phase, the C.M. potentials reduced gradually reaching the maximum decrease 8 to 15 minutes after the apposition of the NaCl crystal on the round window membrane. This reduction of the CM potentials involved both the high and the low frequencies.

3. In the second phase the C.M. amplitude gradually increased, but it still remained below the initial values. This takes a longer time than that of the reduction of the C.M. amplitude.

4. When the C.M. has reached a certain amplitude, it falls again.

5. Finally we observed a recovery of all the frequencies, initially more rapid for the high frequencies. In other words, we observed that the low frequencies are more affected than the high ones: this is very probably due to an impairment of the mechanical transmission systems of the cochlea (a stiffness increase, as in the first stages of Menière's disease (Fig. 11).

At a certain moment, at the end of the investigation, the amplitude almost reached the initial values for the high as well as for the low frequencies.

The phenomena we observed lasted from 30 minutes for small NaCl crystals to 3 hours for the larger ones. The curve of these phenomena

showed that the high frequencies were more intensely involved than the lower ones It was also characterized by a progressive fall in the amplitude of C.M [1]

C. Action Potential Variations in a Single Deiters' Unit

A further electrophysiological investigation was devoted to the recording of modifications of action potentials registered at the level of the second vestibular neuron during the course of the experiments.

A tracheal cannula was placed in cats and precollicular decerebration performed carotid arteries were occluded for 15 minutes. Microelectrodes with steel needles, sharpened electrolytically to tips measuring 10–20 micron in diameter were used. Recording placement was made with a stereotaxic instrument, using a micromanipulator permitting verly slow penetration. Electrical activity of single Deitersian units was amplified by a preamplifier (Tektronix, mod. 122) and measured by an electronic oscillograph (ETC, mod 21 A) Bromide paper was used for recording

After having recorded the responses of the Deitersian units to cathodal and anodal polarization of the labyrinth, a small NaCl crystal was placed on the round window membrane.

After the apposition of the crystal on the round window membrane of the ipsilateral middle ear a single Deitersian unit responding with an increased frequency to cathodic polarization (and with reduced frequency to anodic polarization) of the ipsilateral labyrinth, presented the following variations

1 1 to 2 minutes after the apposition of the crystal, the spontaneous rhythmic discharges increased gradually in frequency and 10 minutes later this increase reached a value three times greater than the initial one simultaneously the extension tonus of the ipsilateral limbs increased as well as the amplitude and the frequency of the potentials from the ipsilateral triceps We observed also a tonic deviation of the eyes towards the opposite side, together with ocular nystagmus

2 15–20 minutes later the rhythm became irregular and discharges began to decrease in frequency

The rigidity phenomena of decerebrated animals at the level of the ipsi lateral forelimbs, and the frequency and amplitude of the electromyogram, were reduced, with irregular groups of discharges The tonic deviations of the eyes were not constant and difficult to observe

3 After 20 to 30 minutes, the electrical activity of the Deitersian units dropped The extensor tonus of the ipsilateral limbs was remarkably reduced and the electromyogram showed no electrical activity of the muscle The eyes were deviated ispilaterally to the ear under experiment, with occasional nystagmus

4 After this period lasting from 20 to 30 minutes, we observed a slow

[1] This investigation was performed by G Molinari, M.D

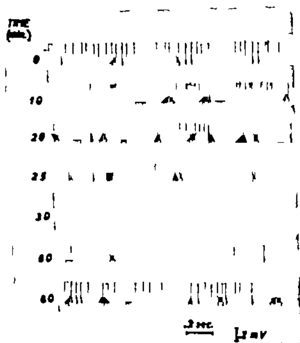


Fig. 12. Modifications of the lectrophysiological activity f singl it of Deiters nucleus during the experiment. 0 before the experiment began (pontaneous activity of the unit) 10 10' after NaCl crystal pposition increasing activity (1 t phase irritativ igns) 20 20' fter pposition, etc. decrease of the preceding discharg ratio. 30 30' fter pposit n, le.t disappearance of activity (2nd phase "paralytic" est. signs) 60 60' after pposition, etc. reappearance f littl acti ty 80 80' after pposition reappearance f spontaneo activity as t 0

recovery of electrical activity of the vestibular nuclei. During this period the electromyogram of the ipsilateral triceps returned to the initial values, while the eyes returned to the normal position. After 80 minutes, the frequency of discharges presented the same values recorded before the in vestigation (Fig. 12)

D Ions and Osmotic Pressure Variations

Cats were used for our experiments, allowing an easier exposition of the round window m mbrane A sodium chloride crystal was placed on this membrane After three minutes this crystal was completely dissolved by the liquid lea ing the inner ear The whole surface of the middle ear was throughly washed and dryed Samples f the scala tympani perilymph were collected by piercing th membrane with a dry glass micropipet carried on microm nipulat r Endolymph from th scala media was collected with a mall r micropipet, after removal of the round window membrane, by piercing the basilar membran in it basal turn.

Thi investigation was performed by G. M Linari, M.D.

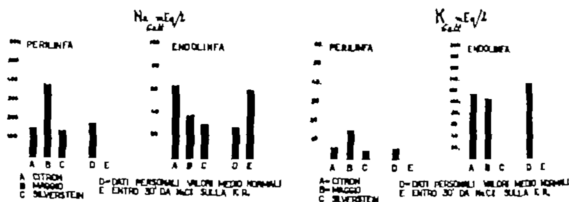


FIG. 13. Cont nt of Na and K ions in the perilymph and endolymph. A B C, data after Citron, M ggio, and Silverstein D personal data. Valori medionormali = middle alues ntro 30' da NaCl ulla F R = values obtained in the first 30' after pposit n of a N Cl crystal on the round window

The microchemical study of sodium and potassium concentration of endolabyrinthine fluids was performed by spectrophotometry with athomic absorption.

Mean values of Na^+ and K^+ ions, both in the normal perilymph and in the endolymph of the cat (see Fig 13) were quite similar to those referred to by Citron and Silverstein

Samples collected within half an hour after sodium chloride crystal apposition on the round window membrane showed a very high increase of sodium ions concentration in the perilymph and a less pronounced increase in the endolymph (Fig 13)

As what concerns the mean values of K ions in the perilymph and endolymph and their modifications after sodium chloride deposition on the round window we observed that the increase of concentration was very high in the perilymph but in the endolymph it was very small (Fig 13)

In Fig 14 we have represented the ratio between endo- and perilymphatic sodium and potassium ions

We observed that the value of the sodium ratio was not modified by

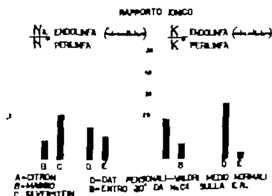


FIG. 14 I na rati f N nd K betwee end lymph and perilymph (translations f Fig 13)

Table 1 *Osmotic pressure relative to Na and K ions.*

$$\pi = \pi Na + \pi K \quad \pi = gcT$$

π Osmotic pressure, g = coefficient, c = concentration, T = absolute temperature.

	Torr	% NaCl
Normal ionic concentrations		
Perilymph	3044	1.048
Endolymph	2503	0.860
Ionic concentrations after 30' NaCl on the round window		
Perilymph	8885	3.053
Endolymph	3646	1.253
% increase of the osmotic pressure		
Perilymph		191
Endolymph		45

sodium chloride on the round window whereas the potassium ratio was profoundly modified, varying from a normal value of 25 to a value of 3.

Variations of osmotic pressure were measured in the endolymph and perilymph related to sodium and potassium ions concentrations. Normal values were quite similar to those reported by Aldred *et al.* (1940)

After sodium chloride deposition on the round window osmotic pressure increased by 191% in the perilymph and 45% in the endolymph (Table 1)

These results call attention to three principal phenomena

1 Global increase of the ionic concentration and consequently of the osmotic pressure

This is connected to a water subtraction from endolabyrinthic liquids. This water through the round window membrane, passes from a less concentrated solution to a more concentrated solution produced by the sodium chloride crystal.

2. Higher *concentration* of sodium in the perilymph than in the *endolymph*. It is possible *to explain* this phenomenon *by admitting that* a small quantity of sodium chloride placed on the *round* window passes into the perilymph of the scala tympani.

3 *Increase* of the potassium concentration in the perilymph, while in the endolymph the concentration is near to normal

It is possible to explain this phenomenon by admitting a potassium passage from endolymph to perilymph In *other words, the increase* of potassium concentration in the endolymph, *resulting* from water subtraction, is cancelled by subsequent passage of potassium into the perilymph. Table 2 summarizes hypothetically the succession of phenomena in the 1st phase of the experiment

These i estigations ere rried ut by G. Perin, Ph D nd O. Molinari, M.D.

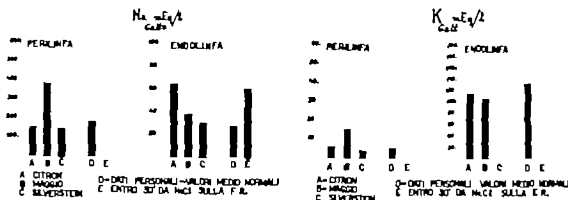


Fig. 13. Content of Na and K ions in the perilymph and endolymph. *A B C*, data after Citron, Maggio, and Silverstein *D* personal data. Val ri medi normali = middle alues *entro 30' da NaCl sulla F.R* = values obtained in the first 30' after apposition of an NaCl cryst l on the round window

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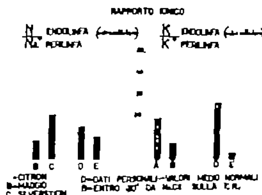


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These investigations re rried out by G. Perin, Ph.D., and G. Molinari, M.D

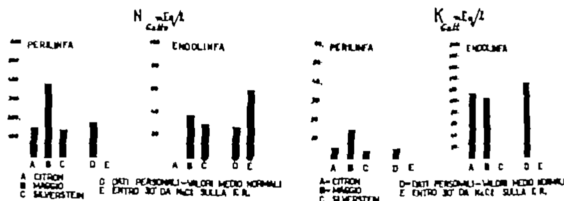


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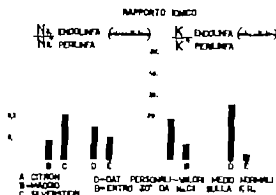


Fig. 14 Ion rati of Na nd K between endolymph nd pe ilymph (tran latio in Fig 13)

of water ultrafiltration increase and decrease of the reabsorption of the *perilymph* and the endolymph) appears fully justified it corresponds to as many stages of *variations of the osmotic balance* between the endolymph and the perilymph.

As a matter of fact, concerning Reissner s membrane, the limbus spiralis, and the other structures, recent investigations with electronmicroscopy (Spoendlin, 1964 Jurato, 1967 v Ilberg, 1968, and others) seem to give confirmation to their permeability

For example, Jurato (1967) states from a morphological point of view that it can be stressed that the extreme thinness of the membrane of Reissner certainly permits diffusion phenomena. Moreover the pinocytic vesicles demonstrated both in the epithelial and in the connective cells, could repre sent some morphological evidence for the passage of fluids through the membrane of Reissner" (page 80)

As in our experiments biochemical factors are neither introduced in the labyrinth cavities nor in the vasal system (blood, lymph, liquoral) it appears evident that only biophysical-induced variations in the labyrinth fluids occur and that they are cause of the observed phenomena.

In fact, we must admit that the regulation of the perilymphatic and endolymphatic function is not only based on biochemical exchanges, but even n biophysical parameters, as osmotic pressure, hydrostatic energy weight, etc. Between the two liquids there is undoubtedly not only a biochemical exchange of ions, molecules, etc., but also some biophysical me chanism which ensures th reciprocal *balance of osmotic* pressure, of hydrostatic energy etc. The permeability of the labyrinth membranes and vessels is certainly an extremely important dynamic biophysical regulating mechanism. Actually an alterati n of thi permeability is followed by a modification of the normal ratio between K ions inside and outside sensory receptors, a we pointed out The alteration of this ratio provokes a dramatic change in the cochlear resting potentials, as the Nernst s equation shows (Molinari, in press)

Analogies of Experim ental Findings and Pathogenesis of Menière's Attacks

A v ry important point of discussion appears to be *the striking similarity existing betwe n tl cour e of these experiments and the clinical picture of the ve tigo ttack in Menière' disease* In fact, if we analyze the pattern and t mporal evolution of the Menière s attacks, in their typical features, we find firstly the presence f "irritative" vestibular signs (nystagmus beat ing to the ill ide etc.) "paralytic signs can be observed too, but with a smaller frequency Furthermore when the attack is ending, p actically only the "paralyt c" igns are to be seen

Moreover the Lerm yez phenomenon(increase of the tinnitus, and some times f the hearing, before the dizziness attack) acquires the meaning of something whi h transitorily "stimulates" the inner ear

TABLE 2 *Phenomena occurring in the first phase of the experiment after NaCl on the round window*

1 Increase in the osmotic pressure of the labyrinthine fluids, which produces
2 Alteration in the permeability of Reissner's membrane followed by
3 Decrease of the ratio between the inner and outer K^+ concentration, which causes
4 Decrease of resting potentials of the labyrinthine receptors, which provokes

Cochlear partition	Vestibular partition
1 Decrease of CM which is responsible together for the increase of osmotic pressure of	1 Increase in the spontaneous activity of the vestibular nerve, which is responsible for
2 Typical deafness due to involvement of transmission system of the cochlea	2 Typical attacks of vertigo irritative nystagmus, etc,

COMMENT

Osmotic balance between the labyrinthine fluids is certainly regulated by the following mechanisms

(1) Secretion and absorption of the fluids with alternative dynamic processes and (2) passage of water ions, and molecules, especially through the Reissner s membrane, ligamentum spirale, prominentia spiralis, vasculo-epithelial zone of Borghesan and other structures (v Ilberg 1968, and others AA)

Concerning the stria vascularis, it performs a double function both of secretion and absorption—this seems to be clearly shown by the electron microscopic investigations of Spoendlin (1964) Reinecke (1968) and others AA)

On the other hand, Rauch (1966) has demonstrated that the whole labyrinthic liquid system (endolymph and perilymph) having a high dynamic metabolism is totally changed in a radial direction nearly every 10 minutes.

The hypothesis that a mechanism of water ultrafiltration, besides a dislocation of ions and proteins, take place in the vessels of the vascular stria and of other secreting structures of the labyrinth, is therefore justified. This process of water ultrafiltration resembles the equal process taking place in the renal tubulus, where the glomerulus secretes nearly all the water and this water is reabsorbed by particular cells soon after reaching the tubulus Then the alternate mechanism of secretion and absorption of the labyrinth fluids could take place also in the labyrinth vessels, since the smallest experimental withdrawal of water from the perilymph causes—as we have seen—important functional variations, both histologic and electrophysiological

As our findings show that the artificial variation of the osmotic pressure of the perilymph provokes functional alterations, first in one direction (irritative syndrome) and then in the contrary one ("paralytic" syndrome) the hypothesis of some compensative mechanisms (sudden increase

Des grandes analogies apparaissent entre l'expériment et les crises aigues du Menière en fait, dans cette crises, les signes irritatives précedent ceux paralytiques. En outre, le collapse du canal endolymphatique qui a été observé dans le Menière initiel et la 1re phase de l experiment, sont les mêmes.

ZUSAMMENFASSUNG

Die Einfuhrung einer gesättigten NaCl-Lösung in das Mittelohr des Meerschweinchens oder der Katze ruft ein spontanes biphasisches Vestibularsyndrom hervor gebildet aus 1 Phase „irritativer" Nystagmus usw 2. Phase paralytischer" Nystagmus usw Der Verf. hat bewiesen, dass dieses Experiment histologische, elektrophysiologische, biochemische, biophysische sehr *interessante* Veränderungen hervorruft, deren Hauptursache der osmotische Druck zu sein scheint.

Es scheinen grosse Analogien zwischen dem Experiment und den akuten Menièrekrisen zu bestehen.

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The fluctuating hearing which is a typical cochlear sign of Menière's disease, clearly indicates, too that the pathogenic processes of this disease are changing moment by moment in their evolution.

The parallelism we point out between our experimental data and the *patterns of the clinical pictures of Menière's disease* means that Menière's attacks perhaps depend on quick alterations of the osmotic balance between the labyrinthine fluids

As a matter of fact, recent biochemical investigations on the inner ear fluids of Menière's patients have shown that there is no variations from normal values (in the uncontaminated samples of perilymph and endolymph removed from the vestibule of these patients) concerning sodium, potassium, and protein content (Schuknecht 1968, Rauch 1966, and others) This fact seems to mean that Menière's attacks do not depend directly on ionic changes of the labyrinth fluids. On the other hand, the hydropic lesions, which are the constant pathologic finding in this disease, are to be considered only *as the final phase of long chain of primary pathogenic factors whose beginning occurred many years before the first attacks* These first pathogenic factors of Menière's disease take place mainly in the delicate ultrafiltration mechanism of water in the labyrinth vessels ultrafiltration is strictly dependent on the permeability mechanism, on the biochemical blood content and so on But the primary pathology in the disease can be, from a clinical point of view silent as the regulation between the perilymph and the endolymph of water ultrafiltration of reciprocal biochemical exchanges, of osmotic balance, etc., can be quickly compensated and return to normality Afterwards, the progressive impairment of the vessels pathology reaches, at a certain moment a loss of the biochemical and osmotic balance at this moment, the first Menière's attack appears in all its clinical patency

The first phase of the experimentally induced loss of osmotic balance corresponds, as we have seen, to a collapse of the endolymphatic duct there are in the literature many findings of this lesion in human Menière's pathology and the "collapse" precedes the formation of bulging lesions of later occurrence.

RÉSUMÉ

L'introduction d'une solution saturée de Chlorure de sodium dans l'oreille moyenne de la cobaye ou du chat, declenche une syndrome vestibulaire spontanée biphasique composée par 1re phase des signes irritatives (nystagmus battant vers l'oreille injecté, etc.) 2me phase des signes paralytiques (nystagmus battant vers le coté non injecté etc)

L A. et ses collaborateurs ont demontré que cet experiment cause des variations hystologiques, electrophysiologiques, biochimiques, biophysiques (variations de la pression osmotique des liquides labyrinthiques) très intéressants. La pression osmotique parait être la cause principale des variations obtenues expérimentellement.

does not support the view depicted by Professor Arslan s histologic specimen for the drastic changes due to sodium chloride application Translent depolarizations by potassium from the endolymph, on the other hand, doesn't produce any histologic changes. A slight bulging of Reissner's membrane in ne turn of the cochlea and a sinking in another is a common artefact which has nothing to do with the distortion of the whole endolymphatic tube which *Kimura* has produced by blocking th endolymphatic duct and sac. *His results seem* to show *hydrop of the labyri th comparable* to that in Ménière cases. As far as I remember kimura has also told me that these animals occasionally have attacks of vertigo comparable to the Ménière attacks. The changes in action potential frequen y shown by Professor Arslan are in accordance with what must be expected from estibular stimulati ns regardless of the mechanism by which it is stimulated.

Mr *Arslan* (Réponse) à Mr *Portmann* 1ère question la microphoniques cochlé ires pendant *la période paralytique rentrent dans la normalité surtout* dan les fréquences aiguës. Ceci est l signe d'une améli ration fonctio elle. 2ème questio j n ai pas f it des expériences uvrant le sac endolymphatique.

To Mr *Friedmann* I did t carry out electromicroscopic controls in our ex periments. But we have planned it.

à Mr *Montandon* Nous n vons pas fait des examens E. N. G. dans nos expérien es. Nous les ferons.

T *Mr Spoendlin* The suggestion is right and we will immedi tely begin the histological researches on the modifications which can appear on th round window membra e after apposition of NaCl crystal. It is quite possible that the com ng out of perilymph water in the middle ear depends from th histological lesions of round window membrane but we payed more attention to the functional effects f the experiences and to the lectrophysiological, biochemical, osmotic ariations, than to the local mechanisms of the outcoming of perilymph.

T M *Dohlman* I agree that it is not possible to identify our experiments w th Ménière attacks. But we believe hypoth tically that by the two different cond tion (the functional *biphasi* estibular spo taneous signs) whi h are the same by Ménière s tta ks a d by the experiments, depend in both from acute osmoti hanges between perilymph and e dolymph. On the other hand we never saw *your histological preparations* a rupture of Reissner' membrane. I gree that the estibular cells and the Corti cells remain normal by cute collapse or successi ely by acut h drops. I lso agree that the hydrop obtained in kimura xperiment is picture whose mechanism is completely different from thi w saw in our experiment In fact, w ha never seen a typical hydrop of cochlea duct, aly "bulgi g" of Reissner' membrane and a compression of the Cort organ, whi h ppears only in the second phase of the estibular ndrome ("paral tic") pro ked by the experiment. We are sure that the hystolog cal p ctures w observ are not artef cts as we compared them with the lab rinth of the pposit side of experimented a imals. I gree that in both situations (kimura experiments nd mine) functional Ménière-like signs, can be observed, but the fact that of ion content don't changes b Ménière patients (Schuknecht, and other AA.) is a aluable argument in fa our f osmotic pathogenesi of Ménière' atta k, as w demonstrated by our experiments.

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DISCUSSION

A Laskiewic I would like to mention that Mr Lindsay and Mr Kimura (Chicago) in 1951 evocated experimentally in animals Ménière-like symptoms by destroying the sacculus endolymphaticus or tightening the ductus endolymphaticus (Kimura) in guinea pigs and rabbits. May I suggest a comparison between the method of Rossem & Arslan with that of the above-mentioned authors, whose experiments have proved in animals the dilatation of the whole endolymphatic system.

M Portmann Très claire démonstration Seulement 2 questions (1) Mr Arslan montre une augmentation du phenomène microphonique pendant la periode paralytique Est-ce dans son esprit le signe d une hyperfonction au point de vue auditif? (2) A t il fait la même expérience après l ouverture du sac endolymphatique ou après intercommunication entre perilymphe et endolymphe?

J Friedmann I wonder whether certain electronmicroscopic studies on his experimental material might assist in confirming our EM-findings, e.g. the presence of intracellular crystalline inclusions and broad-banded collagen

A Montandon L expérience d Arslan qui n a pas la prétention de démontrer le mécanisme exact de la crise de Menière mais seulement d en évoquer l une des possibilités, a le grand mérite de faire intervenir l élément vertigineux, ce que n est pas le cas en général dans l'hydrops expérimental. J aimerais lui demander si en plus de la dérivation des potentiels des fibres nerveuses vestibulaires, il a utilisé l enregistrement ENG qui se rapproche des tests cliniques usuels?

H Spoendlin I would like to ask Professor Arslan whether he has examined histologically the round window membrane during such experiments. I wonder whether only osmotic phenomena play a role in these experiments. We did similar studies in the cat and we found the same labyrinth reaction when we introduced a saturated solution of NaCl but no reaction when we introduced other hypertonic solutions such as solution of saccarose and mannitol.

G Dohlman The experiments which Professor Arslan described are certainly suited for demonstrations to students of vestibular reactions, but I can t see what connection these experiments might have with Ménière s disease As far as I know there has never been shown any evidence of such spectacular osmotic changes or perilymphatic sodium increase in Ménière cases. On the other hand the ruptures in the walls of the distended vestibula sacs which Professor Arslan mentioned seems to me more profitable for a closer study As is well known, the potassium of the endolymph has a concentration sufficiently high to produce a depolarization of the vestibular nerve fibres, as I earlier have been able to show The intact vestibular hair cells even in Ménière cases of long duration

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VESTIBULAR NEURONITIS

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A number of syndromes have been described which are similar to vestibular neuronitis, all with vertigo as their *chief symptom*, in some the vestibular signs and symptoms have occurred alone, in some series evidence of *epidemic nature* was ted, in others neurological changes such as affection of the oculomotor nerve By a follow-up of 60 cases of vestibular neuronitis it is shown to be separate disease entity in which a certain diagnosis can be made. Statistics of age and sex incidence, of the number of atta ks and their duration and severity are tabulated. A severe estibular upset leads frequently to habituation of one or both vestibular systems to stimuli producing an absence of caloric responses in an otherwise normal system. A similar form of habituation occurs in dancers and ice skaters and is associated with certain employments, *thus when the caloric responses are depressed or absent a history cover* i g these possible causes of habituation should be taken.

The condition, now well-known, which was called vestibular neuronitis by Hallpike (1949) cannot be distinguished from Menière s disease by the nature of the vertigo, but solely by the absence of cochlear signs and symptoms. It affects younger adults than does Menière s disease. In a few cases the onset is associated with a febrile illness or an infection in the nose or throat, but as in Menière s disease, infective ear disease does not seem to be a responsible factor The disorder may complicate conditions such as brucellosis (Thompson & Bernstein, 1963) Tinnitus or deafness are notably bsent and the most detailed tests of cochlear function give normal results, but tests of v stibular function are invariably abnormal. Positional nystag mus, type II (benign paroxysmal) (Dix & Hallpike, 1952) or at times type *III* (Harrison, 1966) is often found in vestibular neuronitis and in other severe lesions of the v stibular system such as Menière s disease in which *the v stibular "tonus" is temporarily lost* during changes of position (Hart, 1963)

In most cases the cal ric tests show severe derangement. The degree of di turbance of response depend largely upon the acuteness of the affection and the time between the nset and the test. In *only a few cases does a* unilaterally depressed estibular response persist and when it does, the lesion i almost lways a severe one The difference between *the severe* lesion which continues to produce a marked depression of the caloric responses and the mild r lesion is so remarkable that the two may be different cond tions. In the latter the caloric responses usually show only an abnor

unsteadiness might last several weeks, and especially noticeable on movement, particularly stooping. Following this, 50 personal cases were described by Worster Drought (1952). In some nystagmus was observed. This lasted for several days, but sometimes recurred in a lesser degree during subsequent attacks of vertigo, symptoms resolved slowly usually in 6 to 9 weeks and rarely persisted 12 months. Even with rapid recovery patients were often very unsteady for some time. Walford (1952) suggested influenza as a cause of this type of labyrinthitis and cited an "epidemic" of four cases in which there had been contact with cases of clinical influenza in the same household though examination of the blood showed no evidence of virus infection. A similar series of cases occurred in the U.S.A. (Merifield, 1965)

A large series of cases was reported in which upper respiratory infection was followed by prolonged vertigo (Herberts, 1954). The neurological examination was normal and tests of peripheral vestibular function were normal too. The condition was attributed to a virus encephalitis, a view supported by the finding of an increase of cells and protein in the cerebro-spinal fluid.

Dalsgaard-Nielsen (1953) also described "epidemic vertigo" in which electroencephalographic abnormalities were found in 16 out of 21 of the cases with some correlation with the clinical course of the condition. One case complicated by hepatitis and another by epidemic hiccough, often seen in encephalitis lethargica (Economo, 1929) had normal electroencephalograms. All of Dalsgaard Nielsen's subjects had been in contact with other patients with vertigo and he therefore attributed the condition to an acute cerebral disturbance, probably a virus encephalitis. Nineteen per cent showed hearing loss, though further details of the otolaryngological findings were not reported, but neurological changes were present in 24 per cent, including diplopia in 10 per cent with reflex and sensory changes in others. Twenty-four per cent showed increased sedimentation rates. The cerebro-spinal fluid was normal in the 18 cases in which it was examined.

A similar series of 100 cases of a syndrome closely resembling that of Dalsgaard-Nielsen was reported (Winther) in 1952. The caloric tests were normal in 41 of the 58 patients tested and there were mild changes in the cerebro-spinal fluid in nine out of the remaining 32 cases. The pathological process appeared to be a widespread one thus throughout the central nervous system wit[illegible] estibular symptoms as the predominant and sometimes only symptoms.

The acut[illegible] W[illegible]se (1954) closely resembles the condition [illegible] slower taking up to 48 hours to [illegible] weeks is greater

The large[illegible] lescribed 400 cases in a series of [illegible] n types. Type I— gastro-intest[illegible] r an interval of months after [illegible] tients there was a complaint [illegible] The vertigo was

mal pattern which soon returns to normal Vestibular palsy of sudden onset occurring as a single attack only has been described (Mehmke, 1963) as a separate disease entity A few of these cases may be the result of an occlusion of the vessels supplying the vestibular end-organs from the internal auditory artery in the case of spasm the severity might depend upon the duration of occlusion Hallpike (1949) found that bilateral derangement of the vestibular system was often present, but other authors (Stahle, 1966) state that the condition is invariably unilateral

In many cases the condition is a benign one. The evidence of vestibular disease with the absence of cochlear symptoms and signs was thought to localise the lesion to the vestibular pathways at some point central to the labyrinth, but Hallpike could not specify the particular neurones or neuronal elements which were involved so that the name vestibular neuronitis was left open purposefully

Differential diagnosis (Wlodyka, 1967)

It may be said that although vestibular neuronitis shows some features common to all of the other conditions to which reference will be made in this survey yet the resemblance is no more than superficial Thus, it is distinguishable from the epidemic vertigo of Pedersen by its failure of exhibit any true epidemic characteristics and by the constant finding of abnormalities of the caloric responses

Further the absence in vestibular neuronitis of any neurological abnormalities outside the eighth nerve system clearly separate it from vertigo due to brain stem encephalitis with its associated oculomotor and other neurological disturbances (Poston, 1926 Pedersen, 1959 Leishman 1955) It seems clear that we have to deal with a *number* of nosological entities These have become loosely grouped within a nomenclature which is both inadequate and confusing, and indeed, the terms epidemic vertigo, epidemic labyrinthitis and vestibular neuronitis are often used for any or all of them with little regard to their distinctive clinical and pathological features Clarification of this situation clearly requires that these features should be more clearly specified.

A number of similar syndromes, all pursuing a benign course with vertigo as their chief symptom, have been described in the literature during the course of the last 40 years Vestibular epidemic encephalitis was described by Barré & Reys in 1921 and by Poston in 1926 In some of the cases described vestibular signs and symptoms occurred alone In others oculomotor disturbances were the only neurological abnormalities, accompanied by variable lethargy Encephalitis was postulated as the cause of the condition but no supporting cerebro-spinal fluid data were given

In the past decade several other syndromes have been described, with vertigo as their leading symptom In 1952 attention was drawn in *The Lancet* to the condition epidemic labyrinthitis In this the vertigo was continuous or paroxysmal gradually disappearing in 2 or 3 days, though mild

TABLE 1 *Age incidence of some different forms of vertigo*

	0–20	21–30	31–40	41–50	51–60	61–70	+70	Total
Menière's disease	17	40	95	152	161	85	23	573
Vestibular neuronitis	4	10	34	28	19	11	2	108
Positional nystagmus								
Type I	1	8	12	26	6			53
Type II	3	18	24	65	74	33	3	230
Type III				2	3	1		6
Acoustic neurofibromata	4	27	40	50	33	3		157

The figures of acoustic neurofibromata are taken from C. H. Edwards & J. H. Paterson, *Brain*, 74 part 2, 144 (1951)

Age and sex

The condition is seen most often in the fourth decade and two-thirds of the cases occur in the age group 30 to 60. Thus the peak appears in a younger age group than Menière's disease. Table 2 shows that it occurs more frequently in males.

Electronystagmography

Aschan & Stahle (1956, 1957) find that the electronystagmograph plays an important part in the diagnosis of vestibular neuronitis, showing spontaneous or positional nystagmus even in cases where it is not visible to the naked eye and so facilitating interpretation of the caloric test.

Pathology

Vestibular neuronitis is not a fatal condition so that in no case has the histology been reported, but the findings in a patient with vertigo due to sudden unilateral loss of vestibular function without cochlear symptoms were reported in 1956 (Lindsay & Hemenway). The lesion consisted of degeneration of part of Scarpa's ganglion and of the nerve to the utricle,

TABLE 2. *Age and sex incidence of vestibular neuronitis*

	0–20	21–30	31–40	41–50	51–60	61+	Total
Males	2		17	21	10	4	61
Females	2	3	17	7	9	9	47
Total	4	10	34	28	19	13	108

attributed to dehydration and intoxication. Six patients showed spontaneous nystagmus The eighth nerve function was tested in two cases only the responses were normal in one and there was directional preponderance of the caloric responses in the other Type II—upper respiratory vertigo was present in all the 50 cases and in seven was characteristic of the benign paroxysmal positional type (Dix & Hallpike, 1952 Harrison, 1956) No mention is made of the results of any detailed tests of eighth nerve function However some vestibular tests were carried out in 16 of the severest cases of the two groups who were admitted to hospital and it is noteworthy that although in all of these vertigo was a prominent symptom, yet the tests gave normal results in all but three cases. The disorders described by Pedersen therefore differ from the vestibular neuronitis of Dix & Hallpike in two obvious ways Firstly in their epidemic character which does not appear to be a feature of vestibular neuronitis Secondly and more important the infrequency and trifling character of any evidence of organic vestibular derangement as indicated by the results of the caloric tests. In contrast with this Dix & Hallpike found the caloric responses abnormal in 100 per cent of cases with vestibular neuronitis In spite of the negative results in his cases of the vestibular tests, Pedersen argued in favour of an organic affection of the vestibular tracts within the brain stem, agreeing with the views of Dix & Hallpike.

Epidemics of vertigo have been described from Melbourne from time to time (Williams, 1963) with rising serum antibody titres to coxackle virus and an increased number of cells in the cerebro-spinal fluid. The caloric responses in these cases were normal In Sydney (Basser 1964) attacks of vertigo in children of short duration occur at intervals of several weeks, at times for years. The caloric responses were depressed and no serological abnormality was evident

Incidence

Vestibular neuronitis is less common than Menière s disease and positional vertigo Stahle (1966) found that in the Uppsala Clinic Menière s disease was ten times more common but in Great Britain vestibular neuronitis is twice as frequent as in Sweden (Table 1)

Epidemic characteristics

When vertigo affects several members of a fairly localised community it is not unusual for the family doctor to accept this as evidence of an epidemic The condition may be associated with oro-nasal infections and as these tend to occur in certain seasons such as winter and spring (Gramowski, 1964) so vestibular neuronitis may at times assume apparently epidemic character though often the so-called epidemic is comprised of entirely unrelated types of vertigo (Harrison, 1962)

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Females	2	3	17	7	9	9	47
Total	4	10	34	28	19	13	108

TABLE 3 *Numbers of attacks occurring in each of a series of 108 cases of vestibular neuronitis*

1	2	3	4	5	Over 5
55	14	11	6	4	18

the superior and the horizontal semicircular canals on the affected side. A mass of fine convoluted vessels in the internal auditory meatus invading the ganglion of Scarpa indicated vascular occlusion as the cause of the acute process

The vertigo in this case appears to have shown more positional features than is usual in vestibular neuronitis and Lindsay does not consider that the clinical findings should lead to a diagnosis of vestibular neuronitis.

Prognosis

Vestibular neuronitis is benign in the majority of cases but the duration of the disability after the acute vertigo varies widely from a few days to 1 or 2 years or more and is related in most cases to its severity After the severe vertigo a period of unsteadiness ensues, with, at times, shorter and less severe attacks of vertigo (Table 3) The unsteadiness gradually becomes little more than an insecure feeling on rapid head movements It is rare for the unsteadiness to cause prolonged handicap, in these cases the caloric responses remain nearly absent, in many of the rest of the cases little abnormality can be found in the responses to hot and cold caloric stimuli by the end of a year (Table 4)

Table 5 confirms, as would be anticipated, that when the condition is severe it tends more often to be prolonged though 14 per cent of cases with mild unilateral disease are also of considerable duration.

Diagnosis

One of the criteria of diagnosis in the present series has been the absence of cochlear symptoms and signs. Certain peripheral conditions may at times produce only mild cochlear abnormalities (Pfaltz, 1955 Boffi, 1965) but we agree with Hallpike that this should distinguish such lesions from the condition with which we are at present concerned

From the criteria outlined in the initial paragraphs it is possible to make

TABLE 4 *Duration of vestibular neuronitis in 108 cases*

< 1/52	< 1/12	< 3/12	< 6/12	6/12 +
26	28	21	8	25

TABLE 5 *Duration and severity of cases of unilateral and bilateral vestibular neuronitis*

Severe			Moderate			Mild		
			Unilateral					
24 cases			21 cases			28 cases		
Short duration	Medium duration	Prolonged	Short duration	Medium duration	Prolonged	Short duration	Medium duration	Prolonged
6	8	10	6	10	5	21	3	4
			Bilateral					
20 cases			1 case			2 cases		
5	2	13	1	—	—	2	—	—

a reasonably accurate diagnosis but much doubt has been expressed on the ultimate outcome and final diagnosis in cases appearing initially to be vestibular neuronitis. Thus 60 patients who had suffered from vestibular neuronitis from 15 years to 5 years previously that is in all of them the initial attack was more than 5 years before, were reviewed in the out patient department with their unit system notes. Their family doctors were also asked to supply information of other illnesses these patients had contracted since the attacks of vertigo. All these patients derived from a relatively isolated community of approximately half a million persons in two hospital groups. The case notes in the unit note systems of the hospital group contained records of most of the details of these patients hospital attendances. These three lines of approach—the patient, the family practitioner and the hospital notes—provided a comprehensive history of the patients illnesses.

In one of the 60 patients the diagnosis had since been changed to Menière's disease and in one other case the diagnosis was considered doubtful as the clinical findings were not clear cut In none of the others was a change of diagnosis indicated and in none of the patients had any other disease been found which appeared to have a bearing on the diagnosis.

Vestibular habituation

Habituation, the facility of not responding to or regulating repetitive or unwanted stimuli, appears to be of considerable importance in vestibular neuronitis and following a severe vestibular disturbance, most notably one of endorgan type the caloric responses may remain severly depressed for long after the vestibular system has returned to normal. In such cases habituation may well of course have been the result of other causes, sport, dancing or occupation and if the diagnosis of vestibular neuronitis is in any

doubt an enquiry into the patient's history should be made to exclude these causes of habituation. The effects of habituation on the caloric responses may be of long duration. In the present series these responses have in some patients still been absent several years after the initial illness. Severe bilateral depression of caloric responses due to habituation to rotation may be differentiated by rotation tests in darkness or with closed eyes from vestibular neuronitis. Habituation to vestibular stimuli in such persons as figure skaters or ballet dancers is well recognised. Mowrer (1934) and McCabe (1960) were unable to demonstrate a nystagmic response to any form of vestibular stimulation in skaters with eyes open, but if the test were applied in darkness or with eyes lightly closed (Collins, 1966 *a*) vigorous nystagmus occurred which disappeared when the eyes were re-opened in the light. Collins (1966 *b*) found that the speed of the skaters' spin was too great to allow the "visual spotting" (up to 280 r.p.m.), thus after the sudden stop a few beats of nystagmus were seen, rapidly controlled by visual fixation, but dizziness recurred at once if the eyes were closed. Lidvall (1961) and Gramowski (1964) concluded that the vestibular nystagmic response to repeated stimuli, rotational or thermal, decreased with central training and learning acquired when the stimulus is not required by the brain. Using repeated angular acceleration Lidvall (1961) and Collins & Updegraff (1966) showed that the response decline was fairly specific to a practised direction, affected more the maximum intensity than the duration of nystagmus. This ability to control nystagmus and vertigo is easily and quickly acquired and once acquired may be retained for long periods of time (Hood & Pfaltz, 1954; McCabe, 1964, 1966). Patients with peripheral vestibular disorders more easily become habituated and lose their nystagmic response in light to thermal and rotatory stimuli (Caston & Gribenski, 1960), but by rotating such subjects in the dark or with the eyes closed and simultaneously recording the nystagmus it can be shown that their vestibular responses are otherwise normal (Uchytil & Bochenek, 1966; Mertens & Collins, 1967).

Much variation in the responses to identical rotation in the two directions occurs in normal subjects (Ledoux *et al.*, 1963) and several seconds difference in the responses is not necessarily pathological. In many subjects nystagmus can clearly be seen as the eye moves behind lightly closed lids, giving a useful method of differentiating habituation from true bilateral depression of vestibular thermal responses. The ability to fix the eyes may be reduced by mental arithmetic questions or better still Frenzel's spectacles may be worn. The caloric pattern of unilateral vestibular habituation is a directional preponderance rather than the canal paresis (Hood, 1967) which occurs in bilateral lesions.

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ZUSAMMENFASSUNG

Eine Anzahl der vestibulären Neuronitis ähnlichen Syndrome sind beschrieben alle zeichnen sich dadurch aus, dass Vertigo ihr hauptsächliches Symptom ist. In einigen Fällen waren vestibuläre Symptome allein vorhanden und manchmal wurde dann serienweise ein epidemisches Auftreten beobachtet, in andern Fällen fanden wir auch weitere neurologische Symptome, wie z. B. eine Affektion des Oculomotorius. Durch Weiterverfolgen von 60 Fällen mit vestibulärer Neuronitis konnten wir zeigen, dass die vestibuläre Neuronitis ein abgrenzbares Krankheitsbild darstellt, welches sicher diagnostiziert werden kann. Statistische Tabellen welche Alters- und Geschlechtsverteilung, sowie die Anzahl der Anfälle und ihre Dauer und Stärke enthalten, sind angeführt. Eine schwere vestibuläre Schädigung führt häufig zu einer Gewöhnung eines oder beider vestibulärer Systeme und kann dadurch eine kalorische Unerregbarkeit in einem sonst normalen System bewirken. Eine ähnliche Art der Gewöhnung finden wir auch bei Balletttänzern und Eisschnellläufern, sowie auch in gewissen Berufen. Aus diesem Grunde sollte bei stark verringerter oder nicht auslösbarer kalorischer Reaktion durch genaue Befragung des Patienten abgeklärt werden, ob diese Möglichkeiten einer Gewöhnung bestehen.

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ADAPTATION TO PROLONGED CONSTANT ANGULAR ACCELERATION

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Two independent groups of normal human subjects were exposed to a number of long-duration (up to 90 sec) relatively high-intensity (3 /sec² 24 /sec²) constant, angular accelerations. Nystagmic decrements during stimulation were clearly evident. The decrements were initiated at about the same time after stimulus onset (28-32 sec) for all accelerations used. The decrements in the nystagmic responses were compared to related findings for both subjective and electrophysiological responses.

Response measures of stimulation of the vestibular apparatus may be organized into three general categories (1) reflexive eye-movements (e.g., vestibular nystagmus and counterrolling) (2) electrophysiological recordings, and (3) subjective measures of angular velocity including both indirect measures such as the oculogyral (Graybiel & Hupp, 1946) oculogravic (Graybiel, 1952) and audiogyral (Arnoult, 1950) illusions, and more direct psychophysical measures such as numerical magnitude estimation (Brown, 1966) and key press estimates (Guedry 1965) of velocity

One of the most meaningful approaches to the understanding of vestibular function is the systematic comparison of these different responses during similar stimulus manipulations (e.g. Collins & Guedry 1962 Crampton, 1965, 1966) This approach provides an emphasis on interactions of the vestibular system with other sensory systems, thus facilitating integration and meaningful application of data, as well as defining the most important future research problems. For example although the various intersensory illusions resulting from exposure to accelerative stimulation have been extensively studied, the pervasive influence of vestibular stimulation upon other sensory systems responses does not appear to have been generally appreciated. However when electrophysiologic, nystagmic, and subjective responses to accelerative stimulation are jointly evaluated, it appears that the cerebellum is intricately involved in both the organization of vestibular inputs and the integration of vestibular information with other sensory system (Wolfe 1968) Koella (1962, p. 113) points out that "The cerebellum with it intensive supply of vestibular proprioceptive, tactile, optic, and acoustic impulses certainly is a structure uniquely capable of integrating these afferent signals, there is good electrophysiological evidence that convergence of heteromodal volleys does occur in the cerebellum." Clearly determination of the basic mechanisms involved must include an integration of data from these three general response categories.

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& Crampton, 1964) Constant angular accelerations of precise duration and intensity were programmed from control equipment outside the room which housed the accelerator The subject was enclosed in a light tight removable capsule. Electronic voice communication through slip rings permitted con tinuous contact with *S* An adjustable bite-board maintained *S*'s head in a fixed position over the axis of rotation.

Recording

Electrodes were taped near the outer canthi of the eyes with an indifferent electrode placed in the center of the forehead. EOG signals for summated horizontal eye movements were led through slip rings to a Grass-ink writing recorder in the adjacent control room. Eye movement potentials were amplified with a 3 0-sec, RC, time constant and displayed with a 25 mm/sec paper speed For calibration purposes three lights were placed in front of *S* such that the left and right light subtended a 10 visual angle from the center Prior to each *S*'s first trial and again following his last, the lights were turned on. A 10 voluntary movement of the eyes was recorded to obtain a calibration relating actual angular displacement of the eyes to mm of recorded eye movement. Periods of acceleration also were recorded.

Procedure for Experiment 1

All testing was conducted in total darkness and each *S* was instructed to keep his eyes open and generally directed straight ahead. Fifteen *Ss* were given a counterbalanced series of six experimental trials which consisted of six different angular accelerations (1) 3 /sec^2 maintained for 88 sec (2) 5 /sec^2 for 53 sec (3) 8 /sec^2 for 30 sec (4) 12 /sec^2 for 20 sec (5) 17 /sec^2 for 14 sec and (6) 24 /sec^2 for 10 sec. All accelerations were presented ymmetrically around zero velocity from either 20 RPM (8 12 17 and 24 /sec^2) or 22 RPM (3 and /sec^2) in a CCW direction, through zero velocity to 20 RPM or 22 RPM in a clockwise direction. A preliminary trial of 24 /sec^2 and 10-sec duration provided a check for proper functioning of the recording equipment, and served to acquaint *S* with the experimental procedures. The 13 *Ss* were young men 10–22 years of ge with n rmal labyrinthine function. All Ss had been prevl usly ex posed to laboratory angular accelerations and were selected for the present tudy on the bas of their nystagmic records and high motivation. A high le l of arousal was maintained by requiring *S* to accomplish mental arith metic problem during the trials (both successive multiplication and divi sion) Approximately 15 sec before stimulus onset, *S* was assigned a problem which h continued to w rk until asked for the answer and told to relax by the experimenter A minimum of 5 min. of constant velocity was pro g mmed between trial t decrease the confounding of responses by second ary nystagmu from the previous acceleration.

The biophysical model for the semicircular canals, a torsion pendulum analog (van Egmond *et al* 1949) has provided a useful model for evaluating the varied responses to angular acceleration (e.g Meiry 1965 Aschan, 1954) Primary limitations of the model are its inability to handle either the systematic influence of arousal level upon nystagmic responses (e.g Brown & Crampton, 1964 Collins *et al* 1961) or the occurrence of extensive adaptation (a response decline during stimulation) during long-duration, constant, angular acceleration With regard to the question of adaptation, there are apparent discrepancies between subjective and nystagmic responses to angular acceleration. For example subjective responses involving both key press estimates (Guedry 1965) and magnitude estimates (Brown, 1966, 1968 Clark & Stewart 1967) demonstrate extensive adaptation. On the other hand, Collins & Guedry (1962 1967) reported no adaptation in human nystagmic responses to long-duration constant angular accelerations (1 0 and 1 8 /sec²) when arousal level was carefully controlled. Other investigators have reported declines in both subjective and nystagmic responses (e.g. Hood 1961 Mowrer 1935) Collins & Guedry (1962) suggest arousal level as a primary explanation for experimental differences with regard to nystagmic adaptation at low intensities, but these authors also discuss the possible involvement of higher threshold, quicker adapting receptor units to account for adaptation at higher intensity levels than they examined Evidence from electrophysiological recordings from the vestibular nuclei during angular acceleration are ambivalent (Crampton 1965 1966 Jones, 1967) Although the frequency of discharge from some units appeared to decline under prolonged stimulation, most of those sampled did not decline to any significant extent What decrement is evident might simply be a reflection of overshoot in a non-critically damped receptor system (Cappel, 1966) However since these data were obtained under barbiturate anesthesia, the failure of the units to exhibit any significant adaptation also might be attributed to influences of the anesthetic upon central inhibitory mechanisms (Brazier 1954) In any case, in view of the complete loss of nystagmus under barbiturate anesthesia (Fearing & Mowrer 1934) these data are difficult to interpret relative to adaptation

The primary purpose of the present studies was to determine the extent of adaptation in vestibulo-ocular reflex responses to long-duration, relatively high intensity constant, angular accelerations, and to compare the magnitude of the decrements with that found for subjective responses to similar stimuli

METHOD

Apparatus

Rotatory device

The rotatory apparatus consisted of an angular acceleration device, driven about the vertical axis by an electrohydraulic motor control system (Brown

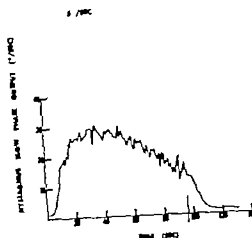


Fig. 1.

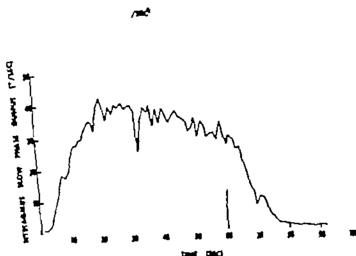


Fig. 2.

Fig. 1-4. Nystagmus slow phase output (/sec) for the four angular accelerations. Vertical bars on the baseline define the time of stimulus termination.

Procedure for Experiment 2

The methods and procedures were essentially the same as that in experiment 1 with the exception that all stimulus durations were maintained as long as feasible within the limitations of maximum RPM. All accelerations were presented symmetrically around zero velocity from 40 RPM in a CCW direction to 40 RPM in a CW direction. Each of 10 experienced subjects received four angular accelerations in a partially counterbalanced order (1) [illegible] /sec² 96 sec (2) 8 /sec² 60 sec (3) 10 /sec² 48 sec and (4) 12 /sec² 40 sec. Arousal level was maintained by mental arithmetic tasks.

Results and Discussion — Experiment 1

Nystagmic responses for all trials except the preliminary trial were scored by hand. The vertical magnitude of the slow phase sweep of each primary nystagmic beat was measured in mm for each 1 sec segment of the record, and then converted to degrees of slow phase by means of the calibration taken at the end of each session. Logarithmic transformations of each *S*'s nystagmic output for each second of every trial were made the logs then summed across *Ss*, and geometric means computed for each second of each of the six trials.

The averaged, second by second, slow phase output is plotted for the six angular accelerations. Adaptation (a decline in output during the period of stimulation) is clearly evident in responses to the longer duration, lower intensity accelerations (3 /sec^2 and 5 /sec^2) Despite the considerable second to-second variation in the nystagmic responses (a typical finding when slow phase is plotted in this manner) lines of best fit drawn by eye for these two accelerations have a negative slope from the points of peak output at approximately 35 sec of stimulation However this decline must be described as only moderate, as compared to the nearly complete adaptation found in subjective estimates of angular velocity to similar accelerations (Brown 1966 1968)

It is interesting to note that the nystagmic decline from peak output was initiated at approximately the same time (35 sec after the onset of acceleration) for both stimuli in spite of the much longer duration of the 3 /sec^2 stimulus. None of the other stimuli were maintained past this apparently critical point for onset of nystagmic adaptation, and no adaptation was evident It would be of considerable significance to determine if the time of onset of adaptation is in fact common to a wide range of angular accelerations To the extent that this could be demonstrated, vestibular adaptation would not appear to be a phenomenon of peripheral origin Adaptation to sensory stimulation has been traditionally viewed as a peripheral not a central phenomenon (Rushton, 1961) But in terms of the mechanical characteristics of the semicircular canals, it is highly unlikely that adaptation to constant angular acceleration reflects changes in the dynamics of the cupula-endolymph system (van Egmond *et al.*, 1949) Löwenstein s work with the ray (1956) indicates that adaptation could have a peripheral locus since different neural units apparently are initiated during cupula deflection and higher threshold units appear to adapt more readily If in fact this were the mechanism for adaptation response declines during prolonged stimulation should be more quickly initiated and have a steeper slope with higher intensity stimuli That is, after 30–35 sec, the cupula would be deviated considerably more and the assumed higher threshold faster adapting units more quickly brought into play for an acceleration of 12 /sec^2 than for one of 3 /sec^2 Experiment 2 was designed to help answer this question.

TABLE 1 *Slopes and intercepts from nystagmic peak*

Output to stimulus termination.

Output to stimulus termination

Acceleration (/sec)	Least squares slope	Least squares intercept	Actual peak output
5	25	30.8* t 32 sec	29.6 at 32 sec
8	25	35.9 at 28 sec	40.5 at 28 sec
10	62	46.4 at 30 sec	48.8 at 30 sec
12	81	51.1 at 30 sec	51.8 at 30 sec

e er it would appear that the onset of the decrement is initiated at approximately the same time following stimulus onset (28–32 sec) apparently independent of the intensity of the stimulus.

In both experiments a nystagmic decrement was evident during acceleration. Further this decrement was initiated at about the same time for all accelerations used and, the more intense the acceleration, the greater the slope of decrement Although it is conceivable that the reported decrements are due to decreases in arousal level during stimulation, as Collins & Guedry (1962) suggest, in view of both the assigned mental arithmetic tasks and the relati ely large decrements found, arousal level would not appear to be a satisfactory explanation of the phenomenon. The manipulation of arousal level evidently can compensate for "adaptation" of nystagmic responses to low intensity accelerations (Collins & Guedry 1962) yet, a decrement is evident despite high arousal level in response to more intense stimuli if the accel ration are maintained past an apparently critical duration of 30–35 sec.

This close time-locked relationship between the onset of nystagmic adaptation and duration f stimulation, which appears to be essentially independent of stimulus intensity is difficult to explain. However the same general time relations are evident in the onset of adaptation for subjective responses (Brown, 1966, 1968 Clark, 1968) i.e., adaptation occurs only after 25–35 sec of stimulation, regardless of the intensity of the acceleration. Both subjective and nystagmic responses appear to be reflecting a generally similar course for onset of a decline during protracted stimulation.

Wolfe (1968) has shown that infra-slow potentials from the cerebellar vermi of c t reflect peaks which may vary in period from as short as 10 sec to a long as 3 minutes. Significant variables appear to be how much stimulation S has recei ed and arousal level. As shown in Fig 3, in cats—there i a slow conjugate shifting of the eyes in addition to the nystagmic response which seem to bear a relationship to these infra slow vermal potential (Wolfe 1968) Slow but systematic phase displacements of the eyes are not uncommonly encountered in human EOG recordings, and,

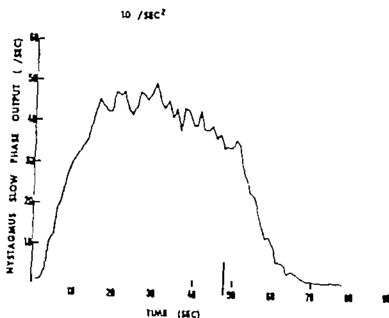


Fig. 3.

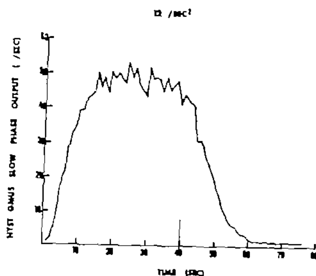


Fig. 4.

Results and Discussions for Experiment 2

Again, geometric means were computed for slow phase output for each second of the four accelerations These data are shown in Figs. 1–4 Several aspects of these data require comment A significant response decline during periods of stimulation is clearly evident in nystagmic responses to each of the accelerations The slope and intercept of each of these four decrements, from actual peak output to stimulus termination, were calculated by the method of least squares. These data are summarized in Table 1 The general trend is for the slopes to become more negative as the intensity of the acceleration increases These data tend to indicate that once initiated, nystagmic output declines more quickly for more intense accelerations. How

RESUMÉ

Deux groupes independantes des hommes normaux comme les sujets d'essai ont été exposées au nombre des accélérations de la longue durée (à 96 sec) de la intensivité relativement élevée (3 /sec²-24 /sec²) constantes et angulaires. Les diminutions nystagmiques pendant la stimulation ont été observées é idemment claires. Les diminutions ont été commencées approximativement le même temps après du commencement de la stimulation (28-32 sec) pour toutes les accélérations employées. Les diminutions dans les réponses nystagmiques ont été comparées vec les resultats relatifs pour les réponses subjectives et électrophysiologiques.

ZUSAMMENFASSUNG

Zwei unabhängige Gruppen von normalen Menschen als Versuchspersonen wurden zur Anzahl von langdauernden (bis zu 96 sec) verhältnismässig höchst intensiven (3 /sec² 24 /sec²) konstanten, winkligen Beschleunigungen ausgesetzt. Nystagmische Verminderungen während der Reizung waren klar offensichtlich. Die Verminderungen wurden ungefähr um dieselbe Zeit nach dem Reizanfang (28-32 sec) begonnen, für alle gebrauchten Beschleunigungen. Die Verminderungen in den nystagmischen Antworten wurden verglichen mit den in Beziehung zueinander stehenden Befunden für subjektive und elektrophysiologische Erwiderungen.

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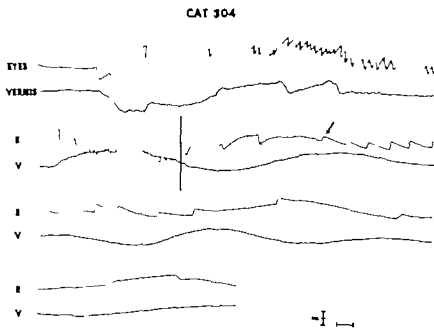


FIG. 5 Record of optokinetic ny tagmus indicating infraslow deviations f the ey s. Note that the period of the infra-slow cerebellar potential closely approximates that of the EOG The arrow denotes th onset of a secondary ptokineti nystagmus. Both the EOG and the cerebellar potential flatten as the optokinetic response drops ut EOG = 500 μv/cm, vermis 200 μv/cm, 3 sec. T C.; 25 mm/sec.

furthermore, Clark & Stewart (1967) have reported a similar "waxing and waning" of subjective responses during long-duration angular acceleration One might speculate that either the vermal potential or the phase displacement of the eyes could provide the necessary neural activity to initiate a response decline Exploration of this intriguing possibility will require com parison of cerebellar potentials and infra slow eye movements with the nystagmic and subjective decrements.

In conclusion, even though nystagmic responses do show a clear decrement during constant angular acceleration yet the vestibulo-ocular reflex does not decline as rapidly or as much as do subjective responses to compa rable accelerations (Brown, 1966 1968) In addition to adapting" more quickly subjective responses also appear to habituate" and recover from habituation more quickly than do nystagmic responses (e g Aschan 1954 Griffith, 1924 Guedry *et al* 1961) In view of the previous discussion it is tempting to speculate further that the response decline during stimulation operationally a classical example of adaptation, is, in fact, only another reflection of a central habituation process.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assistance of D A. Roberts.

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AURAL HARMONICS: THE TONE-ON-TONE MASKING VS. THE BEST BEAT METHOD IN NORMAL AND ABNORMAL LISTENERS[1]

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Amplitude distortion is revealed through the generation of harmonics when a pure tone signal of sufficient intensity is presented to the ear. The psychophysical procedure most commonly used to detect the onset of aural harmonics, called the threshold of the aural harmonic, is the method of best beats. This technique, however, is difficult to use clinically. Complex time-consuming judgements are required and the obtained measurement has an overestimation bias due to masking. A tone-on-tone masking procedure is described and suggested as a simpler alternative for obtaining the same information.

Two experiments have been performed to demonstrate the similarity between the harmonic threshold and the masking threshold. In the first, the thresholds of masking at one octave above the fundamental (= 1000 Hz) and the second harmonic thresholds are shown to be within a few dB SL of each other in normal ears. In addition, both techniques tend to rank the two ears of the same listener in a similar way. The second study shows that the masking thresholds for a small sample of abnormal listeners are lower than for a comparable group of normals. Also, the mean masking threshold of the abnormals, 26 dB SL, is within the range of harmonic thresholds, 13 to 30 dB SL, reported by previous investigators. Thus, these preliminary results indicate that the diagnostic information obtained by the tone-on-tone masking technique is equivalent to the harmonic thresholds measured by the best-beat method. The masking procedure is both simpler and quicker than the best-beat method and, therefore, more practical for use in the clinic.

Pure tone stimulation at sufficient intensities produces perception of the signal's pitch, loudness, and two less directly observable events—harmonic distortion and masking. The ear generates overtones, called aural harmonics (AHs), when the mechanisms within the cochlea (Wever & Lawrence 1954) are forced to vibrate beyond their capacity for simple proportionate response. At the same time the fundamental signal also causes the ear to lose

[1] This investigation was supported by PHS research grant NB 03785 and NB 07242 from the National Institutes of Neurological Diseases and Blindness. Both experiments have been reported before the American Speech and Hearing Association at the annual conventions (1964 and 1967).

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Two experiments have been performed to demonstrate the similarity between the *harmonic threshold* and the *masking threshold*. In the first, the threshold of masking at one octave above the fundamental (= 1000 Hz) and the second harmonic thresholds are shown to be within a few dB SL of each other in normal ears. In addition, both techniques tend to rank the two ears of the same listener in similar way The second study show that the masking thresholds for a small sample of abnormal listeners are lower than for a comparable group f normals. Also, the mean maski g threshold of the abnormals, 26 dB SL, is within the range of harmonic thresholds, 13 to 30 dB SL, reported by previous i estigators. Thus, these preliminary results indicate that the diagnostic information obtained by the tone-on-tone maski g technique is equi lent to the harmoni thresholds measured by the best beat method. The masking procedure is both simpler and quicker than the best-beat method and, therefore, more practical for use i the clinic.

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sensitivity for the higher frequencies. This spread of masking is presumably the reason aural harmonics are not heard as separate perceptual entities at moderate signal intensities even though their presence is detectable using special psychophysical procedures.

The most common of these procedures is the method of best beats, based upon a suggestion by Wegel & Lane (1924) With this method, clinical investigators have measured the lowest intensity (in dB sensation level) of the pure tone fundamental (f_1) required to make the second aural harmonic (f_{AH}) just detectable (for review of logic, see esp Lawrence, 1958) This threshold of distortion has been related to inner ear pathology (Lawrence & Yantis, 1956) to the estimation of cochlear reserve in otosclerotic ears (Yantis & Magielski 1958) to the intelligibility of speech in patients with sensori neural impairments (Yantis *et al* 1966) and possibly even to the determination of susceptibility to acoustic trauma (Lawrence & Blanchard, 1954 Capano, 1962) In spite of its potential significance in hearing conservation and the diagnostic evaluation of hearing disorders, otologists and audiologists do not utilize the best beat method as a regular clinical tool This neglect may be due to certain practical as well as theoretical difficulties One aspect of the latter is the problem of masking at the octave interval and its complicating effect upon resultant measurements.

The confounding effects of sensitivity upon the aural harmonic measurements was first demonstrated and explained by Egan & Klumpp (1951) They suggested that a rather large, somewhat idiocyncratic, bias may be introduced as f_1 intensity is lowered to obtain the aural harmonic threshold. Some more recent work using a different procedure has indicated that the amplitude of f_{AH} is below the perceptual threshold when the ear's distortion begins, and grows at a rate equal to or less-than the masked threshold for intensities below about 70 dB SL (Clack, 1967 1968 *a* 1968 *b*) Such reasoning suggests that the aural harmonic levels, by the best beat method, might be essentially equivalent to the masked threshold measured in the immediate frequency vicinity of f_{AH}

To test this hypothesis, two separate experiments are described here. In the first, the thresholds of masking and the aural harmonic threshold levels were obtained from the same sample of normal ears. The second experiment compares the masking thresholds from a group of sensori neural impaired listeners to the aural harmonic thresholds obtained by previous clinical investigators using the best beat method

I COMPARISON OF THE TONE-ON TONE MASKING AND BEST BEAT THRESHOLDS IN NORMALS

Method

Subjects

Five college students (three male and two female) ranging in age from 22 to 33 with a mean age of 26 years were paid as listeners in this experi

ment. Each subject was given a sweep frequency interrupted tone, Békésy type audiogram. These observers had audiometric thresholds no greater than 25 dB hearing level (re I.S.O., 1964 norms) at octave frequencies between 125 and 8000 Hz.

Best-beats

The subjects hand held the earphone comfortably against the ear being tested. The listener hears a loud, lower pitched, fundamental with a softer higher pitched, background tone which waxes and wanes in loudness, i.e., beats, at a rate determined by the frequency difference between the aural harmonic and the exploring tone. The intensity of the exploring frequency (f) in this case approximately a 2004 Hz tone, is adjusted to maximize the prominence of the beating, obtained initially with a 70 dB SL 1000 Hz fundamental. When the perceptual range of the beat is maximized, as reported by the observer the criterion of "best" is met and the exploring tone is thought to be at the same intensity as the aural harmonic. Both tones were then attenuated together by the experimenter until the beating background could no longer be heard and adjustments of the relative intensity and/or changes in beating rate, due to varying the frequency of the exploring tone, could no longer produce reliable responses. This SL of the fundamental was recorded as the threshold of the aural harmonic.

A Maico Aural Overload Tester MA-3, was used for making measurements by the best-beats method. This apparatus operates effectively as two separate oscillators, one to generate the 1000-Hz fundamental (f) and the other to produce f within a few cycles of the *second* aural harmonic (f_{AH}). The exploring tone can be adjusted separately for "best-beats" at any appropriate intensity of f and both tones can be attenuated together in minimal steps of 5 dB. Pre-experimental calibration showed that the acoustic harmonic output of the associated ear phone was at least 60 dB below the 1000 Hz maximum f_1 output (Lawrence 1958).

Tone-on tone masking

This procedure simply involves measuring the monaural masking at one octave above the fundamental masker. In these experiments, a continuous 1000 ± 1 Hz signal was the masker and the listener traced his threshold for an interrupted 2000 ± 1 Hz test-tone. The apparatus (Fig. 1) consisted of a Hewlett Packard 203A used to generate the f_1 while the test-tone was generated by the Békésy audiometer. Both f and the test-tone were monitored with the electronic counter Hewlett Packard 522B, during the tests to keep frequency drift to a minimum. The matching network consisted of two United Transformer Co. LS-33 impedance matching transformers which prevented any measurable feedback between signals. Earphone output was monitored with the coupler and associated instrumentation. A Brüel and Kjaer (Bk) 4134 microphone, a 2801 power supply and a Hewlett Packard

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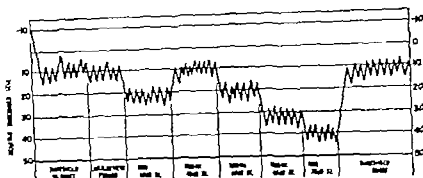


FIG. 2. Typical tracing of the sequence of thresholds obtained from normal ear during one run.

data. After the initial quiet threshold tracing, an interval follows in which the intensity of f is adjusted by the experimenter usually in 10 dB steps, until a masking level is attained which causes a threshold shift of at least 5 dB. In Fig. 2, for example, the f at 60 dB SL produced just over a 10 dB threshold shift. The f was then decreased by 20 dB to find out whether the mere presence of this extraneous stimulus affects the listener's ability to trace his threshold for the exploring tone. The S.D.$_d$ between these tracings and the initial quiet threshold is 1.4 dB. Evidently any threshold shift produced by raising the intensity of the f_1 cannot be attributed to its mere presence. The intensity of f_1 was then increased by 20 dB. The S.D.$_d$ was found to be 1.2 dB between the later and the earlier masked thresholds using this f-intensity. Obviously the small S.D.$_d$'s indicate the reliability of the initial masked threshold levels is quite high. Finally the intensity of f_1 was increased in two steps, usually 5 dB each.

The resulting increases in threshold shift are shown in Fig. 3. These masking shifts appear to grow linearly with only the minor deviations expected in the usual determinations of such thresholds. Using an assumption of linear masking growth, the intensity of f_1 which starts to produce a loss

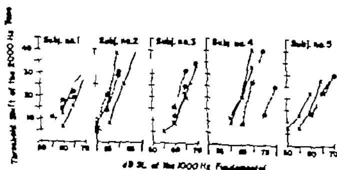


FIG. 3. Threshold shift obtained as the f intensity is increased in 5 dB steps in two separate runs. The masking functions from each of five normal listeners are shown with the dashed lines representing the right ear and the solid lines representing the left ear.

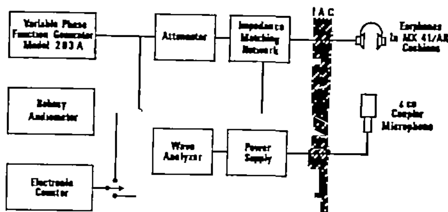


FIG. 1 Electronic equipment used fo calibration and measurement of tone-on-tone masking

302A wave analyzer Any acoustic harmonics were at least 60 dB below the level of f_1

The listener was seated in an Industrial Acoustics Corp (I.A.C.) room and the 1000-Hz threshold was determined in the usual clinical manner using the manual attenuator The listener was then instructed as usual in Békésy type audiometry with the addition of the following "This is the same kind of test you took before (at initial screening) except that the pulsed tone will not change in pitch After a short time you will hear a different tone, one lower in pitch, that will get louder or softer throughout the test. I shall control this tone and you are to disregard it completely Continue to listen only to the first tone which may at times sound more like a chirp in the background. Again, you press the button to make this chirping tone disappear as before. Do you have any questions?"

The threshold tracings for the test tone were converted to numerical values by computing the mean of midpoints of the last 10 pen excursions recorded during each period within a test run

Procedure

Subjects were assigned randomly to either of two testing sequences in which alternate ears of the listener were tested. In addition, the two psychophysical procedures were used alternately in a balanced manner All 10 ears were tested twice by both psychophysical techniques

Results

A tone-on tone masking "run" is illustrated with the tracings of Fig 2 The interrupted 2000-Hz test tone threshold is traced at the beginning and at the end of each run. The standard deviation of differences (S.D.$_d$) was calculated using the 20 "within run" pairs of thresholds. The quiet threshold S.D.$_d$ equals 1 4 dB which indicates that no significant fatigue or practice effect was operating, a fact also obvious from inspection of the raw

ing threshold (TOM) 53 dB SL, is slightly lower than the 59 dB harmonic threshold. Apparently masking at the harmonic frequency becomes effective slightly before there is any evidence of amplitude distortion. This finding is consistent with the bias inherent in the best-beat measurements (Wegel & Lane, 1924 Egan & Klumpp, 1951 Clack, 1967) The absolute magnitude of the difference, 6 dB, is not, however directly comparable to the results of previous investigators for two reasons. First, different instructions or testing details, such as attenuating f_1 and the exploring tone (f) in steps less than 5 dB, might have resulted in harmonic thresholds differing somewhat from those obtained here. Second, an error is involved in determining the threshold of masking from linear extrapolation. It is well known, for instance, that the growth of masking is S-shaped—not *linear* (Wegel & Lane, 1924) Thus, it is quite likely that masking actually begins at lower *levels than* indicated by the TOM values reported here. Although both sources of error affect each of the numerical threshold values to some degree, two facts are clear (1) masking and harmonic distortion appear at almost the same f_1 levels, and (2) both kinds of threshold measurements tend to rank ears in the same way This evidence is consistent with the contention that both psychophysical procedures, tone-on-tone masking and the best-beat method, produce essentially equivalent results in ears with *normal* absolute hearing thresholds. What happens when the absolute threshold is affected by sensori-neural disease processes is investigated next.

II. COMPARISON OF TONE-ON TONE MASKING THRESHOLDS IN NORMALS AND ABNORMALS

Method

All apparatus, calibration, and instructions are essentially the same as in the first experiment. The control group consisted of *eight normal hearing* college students 14 ears were actually tested. The audiometric threshold f these ears were at most 10 dB hearing level (re I.S.O 1964 norms) at octav *frequencies between 125 and 8000 Hz*. The experimental group was composed of eight sensorineural impaired patients (12 ears) obtained from the *outpatient audiology* clinic at the University of Michigan Hospital. Patient selection was based upon a positive otological history evaluation, and a sensori-neural hearing loss of 20 dB or more at three or *more of the* audiometric frequencies tested.[1]

Results

The data needed to calculate the TOM illustrated in Fig 4, was obtained with a "run *modified* from experiment I Each run consisted, first, of a period of tracing the threshold of the test tone (≈ 2000 Hz) in quiet. There

[1] For clinical review f ach patient, see text and appendix.

TABLE 1 *The 2000-H. masking and overload thresholds obtained in the first and second runs from each of five normal listeners*

Subject number	Ear	Tone-on tone		Best beat		Mean thresholds	
		First	Second	First	Second	Tone-on-tone	Best-beat
1	Right	47	50	55	65	48.5	60.0
	Left	54	57	60	65	55.5	62.5
2	Right	48	47	60	60	47 5	60.0
	Left	34	47	60	60	50.5	60.0
3	Right	33	51	53	53	52.0	53.0
	Left	52	56	55	60	54 0	5 .5
4	Right	65	62	60	63	63.5	62.5
	Left	56	56	55	55	56.0	55.0
5	Right	54	52	60	60	53.0	60.0
	Left	51	46	55	55	48.5	55.0
Mean thresholds		53.4	52.4	57.5	60.0	52.9	38.8

of sensitivity in the frequency vicinity of f_{AB} can be quite easily derived from these data The two extreme shifts, the highest and lowest points for each run in Fig 3 are connected by a straight line. The line is extrapolated to intercept the abscissa at some f_1 intensity (This procedure is an expedient made necessary because very slight shifts in the actual threshold tracings cannot be picked out by watching the pen excursions. This simple extrapolation technique removes ambiguity in designating the f_1 intensity which just begins to cause some masking shift of the test tone) The intercept value is defined throughout the rest of this report as the "threshold of octave masking (TOM)

Table 1 permits comparison of all the measurements obtained in each ear of each subject by both psychophysical methods. By comparing the two kinds of thresholds between the ears of individuals, it is evident that a lower TOM tends to coincide with a lower best beat threshold i.e., both measures rank relative thresholds similarly The largest test retest difference between aural harmonic thresholds by beat beats is 10 dB while TOM has a range of 7 dB In 8 of 10 ears ($p < 09$) the TOMs are below the best beat thresholds The mean TOM is about 53 dB for the tone-on tone technique or about 6 dB below the mean obtained by best beats

Discussion

The mean thr shold of the aural harmonic, 59 dB SL, is within the range 52 to 65 dB reported by previous investigators (Lawrence & Yantis, 1956 Lawr n e & Blanchard, 1054) This sample of normal ears, therefore, seems fairly typical of the population. The mean tone-on tone mask

ing threshold (TOM) 53 dB SL, is slightly lower than the 59 dB harmonic threshold. Apparently masking at the harmonic frequency becomes effective slightly before there is any evidence of amplitude distortion. This finding is consistent with the bias inherent in the best-beat measurements (Wegel & Lane, 1924 Egan & Klumpp 1951 Clack, 1967) The absolute magnitude of the difference, 6 dB, is not, however directly comparable to the results of previous investigators for two reasons. First, different instructions or testing details, such as attenuating f_1 and the exploring tone (f) in steps less than 5 dB, might have resulted in harmonic thresholds differing somewhat from those obtained here. Second, an error is involved in determining the threshold of masking from linear extrapolation. It is well known, for instance that the growth of masking is S-shaped—not linear (Wegel & Lane, 1924) Thus, it is quite likely that masking actually begins at lower levels than indicated by the TOM values reported here. Although both sources of error affect each of the numerical threshold values to some degree, two facts are clear (1) masking and harmonic distortion appear at almost the same f_1 levels, and (2) both kinds of threshold measurements tend to rank ears in the same way This evidence is consistent with the contention that both psychophysical procedures, tone-on tone masking and the best beat method, produce essentially equivalent results in ears with normal absolute hearing thresholds. What happens when the absolute threshold is affected by sensori-neural disease processes is investigated next.

II. COMPARISON OF TONE-ON TONE MASKING THRESHOLDS IN NORMALS AND ABNORMALS

Method

All apparatus, calibration, and instructions are essentially the same as in the first experiment. The control group consisted of eight normal hearing college students 14 ears were actually tested. The audiometric threshold of these ears were at most 10 dB hearing level (re I.S.O 1964 norms) at octave frequencies between 125 and 8000 Hz. The experimental group was composed of eight sensorineural impaired patients (12 ears) obtained from the outpatient audiology clinic at the University of Michigan Hospital. Patient selection was based upon a positive otological history evaluation, and a sensori-neural hearing loss of 20 dB or more at three or more of the audiometric frequencies tested

Results

The data needed to calculate the TOM illustrated in Fig. 4, was obtained with a run" modified from experiment I Each run consisted, first, of a period of tracing the threshold of the test tone (≈ 2000 Hz) in quiet. There

For linical review of each patient, see text nd appendix.

TABLE 1 *The 2000-Hz masking and overload thresholds obtained in the first and second runs from each of five normal listeners*

Subject number	Ear	Tone-on tone		Beat-beat		Mean thresholds	
		First	Second	First	Second	Tone-on-tone	Beat-beat
1	Right	47	50	55	65	48.5	60.0
	Left	54	57	60	65	55.5	62.5
2	Right	48	47	60	60	47.5	60.0
	Left	54	47	60	60	50.5	60.0
3	Right	53	51	55	55	52.0	55.0
	Left	52	56	55	60	54.0	5[illegible].5
4	Right	65	62	60	65	63.5	62.5
	Left	56	56	55	55	56.0	55.0
5	Right	54	52	60	60	53.0	60.0
	Left	51	46	55	55	48.5	55.0
Mean thresholds		53.4	52.4	57.5	60.0	52.9	58.8

of sensitivity in the frequency vicinity of f_{AH} can be quite easily derived from these data. The two extreme shifts, the highest and lowest points for each run in Fig. 3, are connected by a straight line. The line is extrapolated to intercept the abscissa at some f_1 intensity. (This procedure is an expedient made necessary because very slight shifts in the actual threshold tracings cannot be picked out by watching the pen excursions. This simple extrapolation technique removes ambiguity in designating the f_1 intensity which just begins to cause some masking shift of the test tone.) The intercept value is defined throughout the rest of this report as the "threshold of octave masking" (TOM).

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Table 2. *TOMs obtained in two successive runs on each ear of eight normal listeners.*

Normal group

Subject	Ear	First run, dB	Second run, dB
1	R. E.	47	47
G. S.	L. E.	48	47
2	R. E.	45	45
C. C.	L. E.	43	45
3	R. E.	47	43
B. B.	L. E.	43	46
4	R. E.	44	44
R. R.	L. E.	48	45
5	R. E.	48	48
E. S.	L. E.	—	—
6	R. E.	51	53
C. H.	L. E.	58	58
7	R. E.	45	45
J. S.	L. E.	55	57
8	R. E.	46	46
T. M.	L. E.	—	—
Mean		48	48

masking tends to grow either faster or slower in the impaired ear than in the normal. Nonetheless, the mean slope ($\overline{M}$) for the entire group of abnormals, 1.7 dB, is quite similar to that of normals, $\overline{M} = 1.8$ dB.

The linearity of the masking function, illustrated for three abnormals in Fig. 5, is evident in all the abnormals regardless of the growth rate. Thus, the TOM can be approximated for the abnormals by linear extrapolation. Again, extrapolation is based upon the two extreme masking values as illustrated in Fig. 5, and the TOMs are defined as the intensity of the 1000-Hz (f) at which the lines intercept the abscissae. The TOMs obtained in the first and second runs of the normals are presented in Table 2.

The reliability of the TOMs among the normals is obvious from a simple comparison of the first and second runs. The largest difference is only 3.0 dB. The mean thresholds for all 14 ears is 48 dB. This mean is 5 dB lower than the 53 dB derived from the normals measured in the first experiment. Such differences between the means probably reflect simply the individuals sampled since the basic procedures are fairly similar. It is interesting to note, however, that this sample produced masking thresholds about 11 dB (59–48) below the aural harmonic thresholds obtained in experiment I.

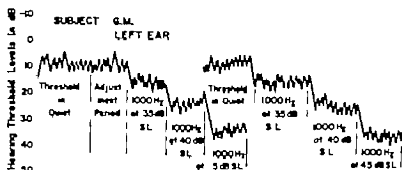


Fig 4 Typical tracing of the sequence of thresh lds obtained from an abnormal ear in each f two successive runs.

follows an interval during which the experimenter increases the 1000 Hz f_1 to produce a threshold shift of at least 5 dB The f_1 intensity was then increased in two steps, usually 5 dB each. Finally the entire run was repeated with the identical sequence of f_1 intensities The S.D.$_d$ for the masked thresholds at each successively higher f_1 intensity level was 2.5 (in quiet) 2 9 2 9 and 2 7 dB for the normals and 1 8 (in quiet) 3.5 2.1 and 2 8 dB for the abnormals, respectively The test retest reliability of the masked and quiet threshold for each group is quite good. Also, the reliability for the abnormals approximates that of the normals.

The masked threshold shifts for three selected ears are presented in Fig 5 These specific threshold shift data were chosen because they span the range of masking slopes (Ms) which, when averaged over both runs for all 12 abnormal ears, were 0 7 0 8 0 8 0 8, 1.2, 1.2, 1 6 1 9 2.5 2 7 2.8, and 3 0 dB These slopes can be compared with those from the 14 normal ears which produced M values of 1 0 1.2, 1 4 1 6 1.8 1 8 1 8 1 8 1 8 1.8, 2 0 2 0 2 6 and 2 6 dB Notice that better than half (7/12) of the abnormal ears have M values outside the range (1 0–2 6) of slopes obtained in this sample from normal ears These distributions of M values suggests that

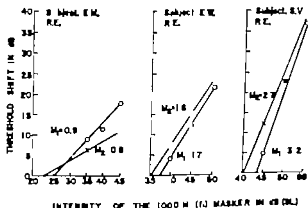


Fig. 5. Thresh ld shifts obtained as the f int sity is incre sed i 5 dB st ps M nd M are the sl pes of two successi runs in the right r (R. E.) of each f three abn r mal li t n rs.

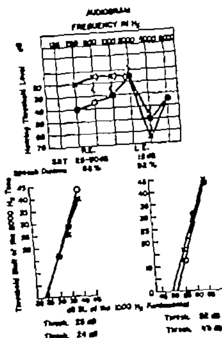


Fig. 6. Preliminary audiometric data on subject R. K. together with the tone-on-tone masking data and analysis.

when the right ear was suffering the exacerbation characteristic of Menière's syndrome, the alternate binaural loudness balance (ABLB) showed the presence of recruitment, and short increment sensitivity index (SISI) scores greater than 60% were obtained at all frequencies tested. The important point here is, however that at the initial visit such evidence of cochlear involvement was not obtained. Although the low frequency thresholds, together with the speech reception thresholds (SRTs) suggest greater involvement in the right ear as compared to the left ear the speech discrimination tests fail to confirm this implication at super threshold levels. The tone-on-tone masking thresholds, however are approximately 25 dB SL in the right and 50 dB SL in the left. Thus, the TOM procedure clearly differentiates between the functioning of the two ears, even when the more usual speech discrimination testing does not.

There is a distinct practical advantage in substituting the TOM procedure for the best beat method. Often the initial best-beat determinations have taken us as long as 30 to 45 minutes, depending upon the experience, motivation, and intelligence of the listener as well as the experience of the tester The listener's task is psychophysically quite complex in attempting to maximize the perceptual loudness range of a soft beating tone. In the tone-on-tone masking situation, on the other hand, listeners only determine whether

TABLE 3 *TOMs obtained in two successive runs on eight abnormal listeners*

Abnormal group

Subject	Ear	First run, dB	Second run dB
1	R. E.	25	24
R. K.	L. E.	52	49
2	R. E.	18	9
R. W	L. E.	22	23
3	R. E.	57	58
G M.	L. E.	30	32
4	R. E.	25	20
E. M.	L. E.	No test	No test
5	R. E.	No test	No test
L. R.	L. E.	2	0
6	R. E.	38	36
E. W	L. E.	No test	No test
7	R. E.	36	35
L. B.	L. E.	31	29
8	R. E.	43	41
S. Y	L. E.	No test	No test
Mean		27	25

Table 3 presents the masking thresholds derived from the first and second runs of all the abnormal ears There appears to be reasonable reliability among the abnormals With the exception of two ears, the thresholds differ by 3.0 dB or less on the test retest basis.

Discussion

The mean aural harmonic thresholds, reported for groups of abnormals where $f_1 = 1000$ Hz, are 13 (Yantis & Magielski, 1958) and 30 dB SL (Yantis *et al.*, 1966) The mean TOM 26 dB SL, obtained with the group of abnormals sampled here falls within this range Also the mean TOM of the abnormals, 26 dB, is clearly lower than the 48 and 53 dB SLs obtained from comparable measurements in normals This lowering of the TOMs suggests their interchangeability with aural harmonic thresholds.

The diagnostic relevance of this substitution is evident in a particularly interesting case R K., a 35 year-old male jet aircraft mechanic The routine audiometric thresholds, seen at the top of Fig 6, indicate a bilateral sensori neural hearing loss caused by noise exposure The symptoms that brought this patient to the doctor are clear cut in this instance—episodic vertigo, vomiting, with tinnitus and pressure in the right ear In a subsequent visit

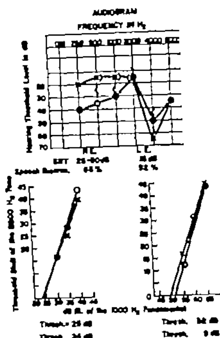


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or not a tone is present. Although extrapolation of results takes a few minutes, the single sets of threshold shifts needed usually requires less than 5 minutes of patient time. Consequently more patients can be tested more quickly than is possible with the best beat method.

CONCLUSIONS

In the first experiment the threshold of octave masking (TOM) was shown to be equivalent to the aural harmonic threshold level in normal ears. The second experiment reveals that even when the ear is impaired by sensori neural disease processes, the TOM is affected in the same way as the aural harmonics threshold. It appears that the results of both experiments provide support for the contention that the best beat method and the tone-on tone masking procedure measure essentially the same phenomenon. Furthermore, in at least one case, it was demonstrated that the TOM is more sensitive to super threshold disfunctioning than the usual speech discrimination test. The tone-on tone masking procedure, therefore, appears to have all the essential diagnostic properties which have made measurement of the aural harmonic threshold desirable. Further studies employing larger samples with a wider variety of hearing disorders, however will be needed before a confident judgement can be made about the clinical value of the tone-on tone masking procedure.

ZUSAMMENFASSUNG

Die Amplitudverzerrung offenbart sich durch die Erzeugung von Obertönen, wenn ein reines Tonsignal von genügender Stärke dem Ohr zugeleitet wird. Der psychophysikalische Vorgang, der meist angewendet wird, um den Beginn von hörbaren Obertönen, genannt die Obertonschwelle, festzustellen, ist die Methode „Best-beats" Die Methode der Best beats ist jedoch in der klinischen Audiometrie schwierig zu benutzen. Komplexe, zeitverzehrende Urteile sind erforderlich, und die erhaltenen Messungen neigen zur Überschätzung aufgrund der Verdeckung. Als ein einfacherer Alternativ Vorgang zur Erlangung der gleichen Ergebnisse wird ein Ton auf Ton Verdeckungsverfahren beschrieben und empfohlen.

Zwei Versuche wurden unternommen um die Ähnlichkeit zwischen der Obertonschwelle und der Verdeckungsschwelle nachzuweisen. Im ersten wurde gezeigt, dass bei normalem Ohr die Verdeckungsschwelle für einen Ton um eine Oktave höher als die Grundfrequenz (= 1000 Hz) und die Schwellen der zweiten Obertöne nur wenige dB SL voneinander getrennt sind. Ausserdem neigen beide Techniken zu einer ähnlichen Reihengliederung der beiden Ohren eines Hörers. Die zweite Untersuchung zeigt, dass die Verdeckungsschwellen einer kleinen Zahl abnormaler Hörer niedriger als die einer vergleichbaren Gruppe normaler Hörer ist. Ausserdem ist die Durchschnittsverdeckungsschwelle der Abnormalen 20 dB SL, innerhalb des Obertonschwellenbereiches 13 bis 30 dB SL, wie frühere Forscher berichtet haben. Demnach weisen diese Vorergebnisse daraufhin dass der Kenn-

zeichnende Aufschluss, erhalten durch die Ton auf T n Verdeckungstechnik der Obertonschwellen, gemessen mit der Best-beat Methode, gleichwertig ist. Der Ver deckungsvorgang ist ei f cher und schneller als die Best-beat Methode und deswegen praktischer für klinische Anwendung

APPENDIX

The f llowing is a clinical description, not previously given in the text, of each abnormal listener sampled in e periment II umbers refer to Table 2.

Subject no. 2 (R. W.) is an 18-year-old male, one of three siblings, who have Alport s sy drome. His sensori-neural impairment is mild-to-moderate with fairly good speech discriminati n bilaterally. R. W. has been followed for 13 years at this hospital and has n history f ertigo, tinnitus, or other ear disease

Subject no. 3 (G. M.) is a 41 ear-old male wh presented with a full sensation and decreased hearing in the left ear. Hea lache, vertigo, or drainage was not reported and the physical examination was unremarkable.

Audiometric xamination revealed a mild bilateral sensori-neural impairment w th sharp loss the higher frequencies (4000–8000 Hz) with the left ea slightly worse than the right. Speech discrimination wa good in the right but poor in the left ear. Further assessment of the left ear produced negative SISI scores at 1000, 2000 and 4000 Hz, clear Type II Békésy audiometric result, and no evidence of tone decay. Unilateral Menière s syn lrome was the diagnosis.

Subject no. 4 (E. M.) is a male, 74 years old, with decreased hearing noticed over the previous 10 years. Physical examination was unremarkable.

The audiometri assessment showed a moderate bilateral sensori-neural hearing loss with a thresh ld decrement for frequencies above 1000 Hz. Speech discrimination was poor. The diagnosis was presbyacusis.

Subject no. (L. R.) had a rather sudden onset of hearing loss in the left ear. This 52-year-old male lost all hearing on the left at e time but it gradually returned. He complained of intermittent dizziness as well as the fluctuating hearing. Seven years prior to this examinati n he h d experienced a imilar problem in the right ear. A caloric test revealed vestibular function in both ears.

Audiometri evaluation indicated a severe b lateral sensori-neural impairment which was slightly greater t 4000 and 8000 Hz with the right ear somewhat worse than the left. Speech discriminati n scores were 68% on the left and only 8% on the right. SISI scores at 1000 2000 and 4000 Hz were positive in the left ear nly. A Type II Békésy audiometric configurati was obtained in the left while the right was a Type I. There was no evidence of t ne decay. A diag osis of Menière s sy drome was made for the left ear.

Subject o. 6 (E. W.) i a 63-year-old mal who complained f decreased hearing over 5-year period. Physical examinatio revealed nothing unusual.

Audiometry showed mild bilateral sensori- eural hearing loss with a very sharp drop in the higher frequencies begin ing at 3000 Hz. Speech discrimination was poor bilaterally. Presbyacusis was diagnosed.

Subject no. 7 (L. B.) is a 77 year-old female with progressi hearing loss in the left ear spanni g the previous 10 years. There was no history of ertigo but she did complain of dizziness. Physical examination was unremarkable.

Audiometri evaluatio revealed a mild bilateral sensori-neural loss ccented by

a sharp decrement above 3000 Hz. Her discrimination scores were good bilaterally. Presbyacusis was diagnosed.

Subject no 8 (S V) is a 64 year-old male complaining of daily episodes of unsteadiness for a period of 7 months. He described his problem as "the room spinning when his eyes were open. Unsteadiness lasted from / to several minutes and was precipitated by turning his head to the right. He reported buzzing heard for 5 years and a pressure sensation.

A moderate bilateral mixed hearing loss was obtained with a depression at 4000 and 8000 Hz. Speech discrimination was fairly good in both ears. The diagnosis was vascular occlusion of the vestibular end artery or viral labyrinthitis.

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THE MIDDLE FOSSA APPROACH FOR REMOVAL OF SMALL ACOUSTIC TUMORS

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We have described a technique for the removal of small acoustic tumors confined to, or slightly protruding into the posterior fossa. The case reports of 5 patients have been presented. Total removal of an acoustic tumor was accomplished in all of these patients. The facial nerve was preserved in 5 of the patients and hearing was saved in 4 patients. The study emphasizes the significance of the complaints of vertigo unsteadiness, tinnitus and mild hearing loss. Patients with these symptoms, either singly or in combination, should receive thorough otologic and radiologic evaluation if they are to receive the maximum benefit from modern neuro-otologic surgery

The great majority of acoustic tumors arise from the vestibular division of the eighth cranial nerve. Only rarely does the tumor originate from the cochlear division (Crow & Hardy 1936 Skinner 1929) Cochlear deficit results from the pressure by the tumor in the limited confines of the internal auditory canal. We have felt that if the diagnosis of an acoustic tumor could be established while the tumor was small before severe cochlear nerve damage tumor removal could be accomplished with preservation or improvement of hearing The present paper reviews our experience in attempting to accomplish this goal, using *a middle fossa approach to the internal auditory canal.*

The middle fossa approch to the internal auditory canal was developed in 1958 (House 1961 1962, 1963) to decompress the internal auditory canal in cases of far advanced otosclerosis. This approach was satisfactory but the decompression had no noticeable effect on the course of the disease

As experience with this approach increased, it became apparent that the operation could be adapted to the removal of tumors situated in the cerebellopontine angle to reduce the disabling morbidity associated with the conventional suboccipital operation.

Between 1961 and 1963, 22 acoustic tumors were approached through a middle fossa exposure. These patients have previously been reported (House 1964) In these cases, a wide removal of temporal bone to the level of the jugular bulb was carried out through the middle fossa approach.

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During the same period several operations were performed through the middle fossa approach for the relief of dizziness. In these cases a wide resection of temporal bone was not necessary since the objective was to section the superior vestibular nerve and to preserve the facial and cochlear nerves. In one of these patients a small resectable tumor was discovered in the internal auditory canal. As a result of this, a basic revision of our concepts concerning surgical exposure of these tumors occurred. We felt that the middle fossa approach allowed direct access to the internal auditory canal without necessarily increasing a neurologic deficit. Preservation of facial nerve function and hearing was possible in patients with acoustic tumors if an early diagnosis could be made.

At that time the perfection of surgical technique for the removal of these tumors had advanced beyond our diagnostic capability. Fortunately this dilemma was soon resolved by the development of accurate radiologic techniques for the demonstration of these tumors using positive contrast material (Scanlan, 1964). Recently the introduction of the polytome x ray technique in association with a small aliquot of contrast material has further augmented our diagnostic capability (Hitselberger & House 1967). The detection of tumors in the internal auditory canal is now possible even when they are a few millimeters in diameter. We feel that these tumors represent the ultimate therapeutic challenge to the neuro-otologist using the middle fossa exposure to the internal auditory canal.

TECHNIQUE OF MIDDLE FOSSA EXPOSURE

Preparation of the patient

The middle fossa approach has been used for removal of small acoustic tumors, facial nerve tumors, and for section of the vestibular nerve in selected cases of advanced Menière's disease. The technique to be described has been used with little modification since 1963.

The operation is performed under light general anesthesia. A supine position is used with the patient's head turned 90° to the side, the involved ear being uppermost. The surgeon is seated at the head of the operating table with the operating microscope on his right and the nurse on his left. A Mayo stand extends over the patient's chest. The anesthetist is positioned at the foot of the operating table. Rubber extension tubing rests on the patient's abdomen and connects the anesthetic machine to the endotracheal tube.

Incision and exposure

The incision extends from a point one finger breadth in front of the tragus at the level of the zygoma, supero-posteriorly to above the helix of the ear. To avoid the fibers of the facial nerve going to the frontalis muscle the incision is kept above the level of the zygomatic arch. A curved incision is preferred to a straight coronal incision to prevent fore shortening of the



Fig. 1 Surgeon view Rectangular shaped bone flap. Note that sides are parallel to facilitate placement of Urban-House retractor

operative exposure. Blunt finger dissection is used to develop the plane between the scalp and temporalis muscle, palpating the root of the zygoma and the zygomatic arch. The temporalis muscle is incised along the direction of its fibers from the zygomatic root to its attachment along the supratemporal line. Additional exposure is obtained by incising the temporalis muscle at right angles to the direction of its fibers along the zygomatic root giving an inverted T shaped appearance to the muscle incision. The superficial temporal artery is usually opened and bleeding is controlled by coagulation or suture. Muscle and periosteum are elevated from the underlying calvarium, and self retaining retractors are positioned beneath the muscle.

A rectangular shaped temporal bone flap is turned using the dental drill with cutting burr. A bone flap is preferred over a craniectomy to avoid a cosmetic defect postoperatively. It is important that the opening be rectangular and that the sides are parallel. This facilitates the placement of the Urban House retractor which will be used to elevate the dura overlying the temporal lobe. The base of the bone flap should lie slightly above the zygomatic root, close to the floor of the middle cranial fossa. Its dimensions should be one-third in front of the external auditory canal and two-thirds behind this point. The superior border is at the level of the squamous suture of the temporal bone (Fig. 1).

The draped operating microscope is brought into the field and is used



Fig. 2 The floor of the middle fossa Retracto bl de elev ting th dura verlying the temporal lobe Th n urovascular structures have been exposed. The middl m i geal artry li nterio ly Behind thi the great superfi ial petrosal n rve ha bee unroofed post rio ly to the g niculate ganglion. From thi point furth unroofi g has been carried ut t expose th internal audit ry can l Not the positi n of the carotid artery sigm id si us, and semi-circul ca ls.

during the remainder of the procedure The Urban House retractor is secured to the sides of the opening, in the epidural space Care is taken not to puncture the dura or open branches of the middle meningeal artery The construction of the blade retractor assembly using built in gear trains, allows elevation of the dura without sacrificing manoeuverability of the blade in either an anterior or posterior direction

After elevation of the dura overlying the temporal lobe, the first landmark which the surgeon should identify is the middle meningeal artery This is the basic landmark for all middle fossa surgery Once the exact position of this important artery has been found, the location of other structures can be determined Lying anterior and medial to the artery is the foramen ovale and the third division of the fifth cranial nerve Almost directly medial covered by a thin roof of bone, is the eustachian tube. Medial to the eustachian tube is the carotid artery also covered by a thin layer of bone Posterior to the middle meningeal artery is the hiatus for the greater superficial petrosal nerve. Lateral to the artery is the roof of the temporomandibular joint (Fig 2)

After identifying the middle meningeal artery the middle fossa dura is elevated back to the petrous ridge. At the junction of the middle fossa dura and the posterior fossa dura, the superior petrosal sinus will be seen The dura should be elevated forward following the superior petrosal sinus until the arcuate eminence comes into view The hiatus of the greater superficial petrosal nerve can now be identified lying between the arcuate eminence and the foramen spinosum.

Bone removal

The bone above the hiatus and greater superficial petrosal nerve is usually thin In 5% of patients it is absent posteriorly to the geniculate ganglion. This bony covering is removed with the drill using a diamond stone. The greater superficial petrosal nerve is adherent to the undersurface of the dura and should be stripped with a small dural elevator in a posterior anterior direction. This increases the anterior limit of the exposure and allows dural elevation as far anteriorly as the level of the middle meningeal artery

The point of the retractor blade is now placed medial to the geniculate ganglion over the petrous ridge This allows sufficient temporal lobe elevation for further bone removal with the diamond burr After exposing the geniculate ganglion, the position of the superior semi-circular canal is determined. This is exposed with the diamond burr by removal of bone over the anterior aspect of the arcuate eminence There may be a layer of overlying cystic bone and after removal of this, the hard, white bone of the otic capsule will be seen. The semi-circular canal appears as a thin, blue line in the thick bony matrix of the otic capsule. It should be exposed from the superior petrosal sinus laterally to the ampula. Once this has been done the posterior limit of bone removal for exposure of the internal auditory canal has been completed.

The next step in the procedure is the exposure of the lateral extent of the internal auditory canal This is done by uncovering the labyrinthine portion of the facial nerve from the geniculate ganglion to the lateral extent of the internal auditory canal, leaving a thin, protective remnant of bone over the nerve itself

The identification of the triangular piece of bone ("Bill's Bar") at the lateral limit of the internal auditory canal is necessary in order to separate the facial nerve anteriorly from the superior vestibular nerve posteriorly This partition marks the actual junction of the labyrinthine portion of the facial nerve and the superior vestibular nerve. Once it has been uncovered, bone is removed over the remaining portion of the internal auditory canal and porus acousticus.

The internal auditory canal

We are now ready to open the dura overlying the internal auditory canal. An incision is made from the porus acousticus to the lateral extent of the

canal By opening the *dura posteriorly a flap is obtained* which is based along the anterior bony margin of the canal This serves as a protective covering for the facial nerve during the remainder of the operation

The dura is reflected and the superior vestibular nerve is identified entering the ampulla of the superior semi-circular canal This nerve is followed medially until the surface of the tumor is seen The plane between the facial nerve and the tumor can now be developed throughout the extent of the internal auditory canal because positive identification of the free nerve fibers of the superior vestibular nerve has been established prior to dissection of the tumor The surface of the tumor is usually smooth and well demarcated The tumor itself may be firm, soft or cystic Dissection of the tumor form the surrounding nerves should proceed slowly As the inferior pole of the tumor is freed, the cochlear nerve can be seen In this plane will also be seen the internal auditory artery The artery is vulnerable during most of the dissection and care must be exerted to stay close to the surface of the tumor The same technique should be used in separation of the tumor from the artery as had been used in the preservation of the nerves. We must remember that the hearing function is dependent both upon a competent cochlear nerve and an intact arterial supply

After the tumor has been separated in the internal auditory canal any medial extension in the posterior fossa should be removed If a clean line of dissection has previously been developed, the tumor can usually be shelled out of its arachnoid bed in the posterior fossa without damaging surrounding structures Occasionally the lateral branch of the anterior inferior cerebellar artery will contact the surface of the tumor and a small *loop of this artery may actually extend into the internal auditory canal* In these situations the artery must be separated from the tumor using the same careful techniques as have been employed in the internal auditory canal

Closure

After tumor removal has been completed, the operative area is inspected for small bleeding points that are controlled before proceeding to the closure The dural opening is closed using a free temporalis muscle graft This prevents postoperative spinal fluid leakage

The retractor and blade assembly are then removed The middle fossa dura is allowed to re-expand The rectangular temporal bone flap is replaced Temporalis muscle is re-approximated with interrupted suture The skin and galea are closed in one layer using interrupted wire suture

A drain is usually not necessary A dry sterile dressing is applied.

Postoperative care

The patient is observed overnight in the intensive care unit Heavy antibiotic coverage in the postoperative period has not been routinely used

Ambulation is begun on the first postoperative day. Hospitalization usually runs from 7 to 10 days. The wire sutures are removed on the 14th postoperative day.

CASE REPORTS

Five patients with intracanalicular acoustic tumors have been operated upon using the middle fossa approach. The hearing remained normal in two, was improved in two, and was lost in one. All cases retained facial function.

Case No. 1

Summary. Unsteadiness two-and-a-half years prior to surgery. Total removal of acoustic tumor. Middle cranial fossa approach. Preoperative diagnosis, Menière's disease.

History. This 60-year-old housewife began to have unsteadiness two-and-a-half years before surgery. Six months' history of right hearing loss and tinnitus.

Examination. Within normal limits except for findings related to the eighth nerve on the right. Eighth nerve (Fig. 3): SRT 50 dB. PB 30%. Békésy Type IV. SISI 0%–1000 c/s, 0% 2000 c/s. Vestibular: Electronystagmography revealed 100% right canal paresis. Cerebral spinal fluid protein 34 mg %.

X-ray. Normal temporal bones. Iophendylate studies failed to reveal presence of acoustic neuroma on the right.

Surgery. Through a middle fossa approach, the right internal auditory canal was opened. The canal was found to be completely filled with acoustic tumor. The tumor was carefully removed and it was possible to save both the facial and cochlear nerves. The tumor arose from the superior vestibular nerve. The inferior vestibular nerve was deliberately sectioned. The tumor measured 5 × 6 mm. No blood transfusion was necessary.

Postoperative course. The postoperative course was uneventful during the patient's hospitalization. She was discharged on the sixth postoperative day. *There was no facial weakness.*

When seen one month postoperatively, the patient had experienced complete relief of unsteadiness and vertigo. The Békésy had converted from a Type IV to a Type II. The discrimination had improved from a 30% preoperative level to an 80% level at the time of this examination.

Comment. This patient was the first in our series to undergo total removal of an acoustic tumor with preservation of both the facial and cochlear nerves. The principal reason for exploring the internal auditory canal by the middle fossa route was to relieve the patient's vertigo by selective section of the vestibular nerves with preservation of the cochlear division. When a small tumor was encountered, it was removed with preservation of the cochlear nerve. *It is significant that both the plain x rays and the iophendylate study of the posterior fossa were reported to be normal.* Although a

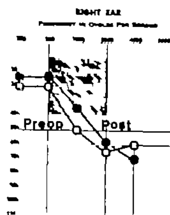


FIG 3

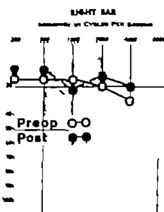


FIG 4

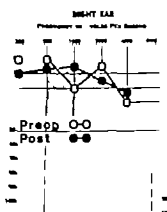


FIG 5

FIG. 3 Case 1 Pre- and postoperative pure tone audiograms.

FIG. 4 Case 2 Pre- and postoperative pure tone audiograms.

FIG 5 Case 3 Pre- and postoperative pure tone audiograms.

dramatic result was obtained here, the case pointed out the need for more accurate diagnostic techniques if we were to recognize these tumors while they were still confined to the internal auditory canal

This patient was last seen in August 1966 Her hearing has remained at an 80% discrimination level The pure tone audiogram has remained similar to that obtained before surgery The patient has been completely free of vertigo unsteadiness and tinnitus since her surgery

Case No 2

Summary Episode of vertigo associated with hearing loss and tinnitus on the right eight months prior to surgery Total removal of acoustic tumor middle cranial fossa approach Previous diagnoses included Menière's disease, vertebral basial insufficiency and multiple sclerosis.

History This 42 year-old physician noted the onset of unsteadiness, hearing loss and tinnitus on the right eight months previous to surgery The hearing loss and tinnitus remained constant The unsteadiness was punctuated by episodes of true vertigo One-month history of dull, aching pain within the right ear

Examination Findings were normal except for those related to the eight nerve Eighth nerve (Fig 4) SRT 50 dB PB, 14% Békésy Type III SISI 0%–1000 c/s, 0%–2000 c/s, tone decay 80 dB–1000 95 dB–2000 Vestibular Electronystagmography revealed 30% canal paresis on the right Cerebral spinal fluid protein 28 mg %

X ray Temporal bones were normal Iophendylate study of the posterior fossa revealed a small predominantly intracanalicular tumor protruding slightly into the posterior fossa.

Surgery The patient underwent a right middle fossa exploration of the right internal auditory canal A total removal of an intracanalicular tumor

arising from the superior vestibular nerve was obtained. The facial nerve and cochlear division of the eighth cranial nerves were spared.

Postoperative course The patient had a moderate right facial weakness immediately postoperative. He experienced complete relief of unsteadiness and tinnitus.

Six weeks postoperatively the pure tone audiogram had returned to normal The patient's discrimination rose to 100% The Békésy converted to a Type 1 The facial paralysis had cleared completely

Comment An interesting observation by this 42 year-old physician prior to his surgery was the fading away of the loudness of the dial tone of the phone—a unique example of tone decay Subsequent to tumor removal, the loudness of the dial tone has been maintained. This patient also demonstrates the apparent susceptibility of the cochlear division to pressure by even a small tumor in the limited confines of the internal auditory canal Note the profound loss of discrimination ability (PB 14%) profound tone decay (9 dB-2000) and only moderate pure tone loss. After tumor removal, the restoration of these audiologic parameters to normal has been almost complete.

Case No 3

Summary One-year history of tinnitus and occasional pain in the right ear Total removal of an intracanalicular acoustic tumor by the middle fossa approach. Excellent preservation of auditory function.

History This 44-year-old female noted the onset of a tinkling sound" in the right ear one year prior to being seen. The character of the sound changed to a buzzing sensation and became continuous. Occasional deep pain in the right ear

Examination Seventh nerve Decreased sensation in the external auditory canal on the right. Eighth nerve (Fig. 5) SRT 20 dB PB, 96% Békésy Type II SISI, 55%-2000 c/s, 95%-4000 c/s. Vestibular Electronystagmography revealed normal responses to caloric stimulation. Cerebral spinal fluid protein 35 mg %

X ray The entire right internal auditory canal was larger than that on the left, and there was definite evidence of a funnel like widening of the internal uditory meatus. These findings were considered diagnostic of an acoustic neuroma on the right

Polytome iophendylate studies revealed failure of filling of the internal auditory anal on the right by the contrast material. There appeared to be a soft ti sue mass present t the uditory meatus blocking the entrance the internal auditory canal (Fig 6)

Surgery The patient underwent a right middle fossa exploration of the internal auditory canal An acoustic tumor was found to fill the entire canal. This was carefully separated away from the f cial, cochlear and superior vestibular nerves. There was no bleeding during the dissection of the tumor The tumor was adherent to the facial nerve and was separated



FIG. 6 Case 3. Polytom l phe dyl te study sh wing f ilure of filling of th right l ter nal audit ry c nal by contra t material A crescent shaped defect is seen t the ditory m tus

away from this with some difficulty The tumor did not extend into the posterior fossa. The estimated size was 5 mm No blood transfusion was necessary

Postoperative course The patient had moderate right facial weakness immediately following the surgery This completely resolved within six months. She experienced some unsteadiness on rapid positional change in the first month after surgery but this has subsided since that time Post operative audiologic examination six months after surgery revealed an SRT of 10 dB and a discrimination score of 100%

Comment This patient has made an excellent recovery following the removal of a small acoustic tumor confined to the internal auditory canal At this time, her hearing is normal in the operated ear and she has no facial weakness.

This patient demonstrates the importance of obtaining petrous pyramid x rays in those patients complaining of unilateral tinnitus.

Case No 4

Summary Three month history of tinnitus and decreased hearing on the right. Occasional light headedness. Total removal of a 6 mm, acoustic tumor with preservation of the superior vestibular and facial nerves. No return of hearing subsequent to the surgery



FIG. 7 Case 4. Polytome lophendyl te study showing failure f filling f right internal uditory canal by contrast mat rial. The tumor is just beginning t p h out l to the posterior fossa.

History This 33-year-old male noted the onset of medium pitched tinnitus, accentuated by fatigue, three months prior to being seen. Associated decreased hearing on the right. One episode of itching in the ear lasting about ne week. Occasional momentary light-headedness.

Examination. The neurologic examination was normal except for those findings related to the eighth cranial nerve. Eighth nerve SRT 45 dB PB, 40 Békésy Type II SISI, 100%–1000 c/s, 100%–2000 c/s. Vestibular Electronystagmography was normal Cerebral spinal fluid protein 82 mg.

X ray Temporal bone x-rays showed some enlargement of the right internal auditory canal with funneling at the meatus. There was slight erosion of the superior lip of the auditory meatus. These findings were considered highly suggestive of an acoustic neuroma on the right.

Polytome lophendylate tudies showed no filling of the internal auditory canal n the right with contrast material. A crescent shaped defect was seen at the meatus. These findings wer considered diagnostic of an acoustic neuroma on the right limited to the internal auditory canal (Fig 7)

Surg ry The patient underwent a middle fossa exploration of the right internal auditory canal. A quote from the surgical dictation is as follows "The dura was opened along the posterior aspect of the internal auditory canal up t the junction of the superior vestibular nerve and the facial nerve The superior vestibular and the facial nerves were then separated by running an instrument between them starting at the bar of bone which



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Case No 4

Summary Three-month history of tinnitus and decreased hearing on the right Occasional light headedness Total removal of a 6 mm acoustic tumor with preservation of the superior vestibular and facial nerves. No return of hearing subsequent to the surgery



Fig. 7 Case 4 Polytome iophendylate study showing failure of filling of right internal auditory canal by contrast material. The tumor is just beginning to push out into the posterior fossa

History This 33-year-old male noted the onset of medium pitched tinnitus, accentuated by fatigue, three months prior to being seen. Associated decreased hearing on the right. One episode of itching in the ear lasting about one week. Occasional momentary light headedness.

Examination The neurologic examination was normal except for those findings related to the eighth cranial nerve. Eighth nerve SRT 45 dB PB, 40% Békésy Type II SISI 100%–1000 c/s, 100%–2000 c/s. Vestibular Electronystagmography was normal Cerebral spinal fluid protein 82 mg.%

X ray Temporal bone x rays showed some enlargement of the right internal auditory canal with funneling at the meatus. There was slight erosion of the superior lip of the auditory meatus. These findings were considered highly suggestive of an acoustic neuroma on the right.

Polytome iophendylate studies showed no filling of the internal auditory canal on the right with contrast material A crescent shaped defect was seen at the meatus. These findings were considered diagnostic of an acoustic neuroma on the right limited to the internal auditory canal (Fig 7)

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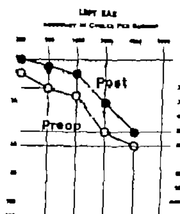


FIG. 8 Case 5 Pre- and postoperati e p re ton udiograms.

separated them The tumor was found lying in the area between all the nerves and it was carefully separated away from the facial superior vestibular and cochlear nerves. A total removal of the tumor was accomplished. Bleeding was encountered from a branch of the internal auditory artery"

Postoperative course The patient made an excellent postoperative recovery He had no facial weakness However hearing was not present subsequent to the surgery and six months following the operation it has not returned

Comment The patient experienced good relief of the tinnitus and light headedness following the surgery However he did not regain any hearing function in the operated ear One can theorize that the damage to the internal auditory artery at the time of surgery resulted in a dead ear It must be noted also that preoperatively hearing impairment in the ear was profound both on the pure tone audiogram and the discrimination test Probably as the neural damage increased subsequent to the enlargement of the tumor in the internal auditory canal, the chances for reversal of a hearing loss lessen

Case No 5

Summary Seven month history of tinnitus. No unsteadiness or hearing loss Total removal of a 3 mm tumor confined entirely to the internal auditory canal with preservation of the facial superior vestibular and cochlear nerves

History This 36-year-old airline pilot noted the onset of fullness and tinnitus in his left ear seven months prior to being seen There was no vertigo unsteadiness or hearing loss

Examination Neurologic examination was normal Eighth nerve (Fig 8) SRT o dB PB 96% Békésy Type II SISI 0%–2000 15%–4000 Vestibular Electronystagmography was normal Cerebral spinal fluid protein 36 mg %

X ray Temporal bone x rays showed a slight enlargement of the left internal auditory canal The meatus appeared normal



FIG. 9 Case . Polytome iophendylate study showing truncated defect i the contrast column at the lateral end f the left internal uditory canal.

Iophendylate examination showed a *small, crescent-shaped defect at* the lateral extremity of the left internal auditory canal. This was consistent with a small mass lesion measuring *not more than 5 mm (Fig 9)*

Surgery A middle fossa exposure of the internal auditory canal was carried ut. *After* pening the dura, the facial and vestibular nerves were seen t be pushed upward and thinned out by an underlying mass. By separating the nerves, a small tumor was exposed in contact with the l teral end f the internal auditory canal. The tumor was mobilized and r moved *en to*. It was not possible to say exactly where the tumor origi nated The main trunk of the superior vestibular nerve was spared and it was *ssumed that the tumor originated from the inferior vestibular nerve.*

Postoperative course The patient had excellent hearing and facial func tion following the surgery He made an uneventful recovery and was placed back n flying status six months postoperatively

Repeat audiometric and vestibular tests were carried out four months postoperatively *These showed an SRH of 15 dB, PB 88%* Békésy Type *II* Electronystagmographic testing was normal (Fig 10)

Comment We anticipate a total *recovery of hearing in this patient. He* demonstrat s a case in which we were able to eliminate, rather than accentu te a neurologic deficit This is significant because f the patient s occupation as an airline pilot. He had been taken off flying status because f minor audiologic complaint nd f r this reason he was most insistent

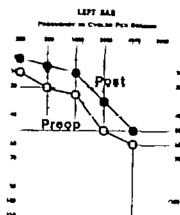


FIG. 8 Case 5 Pre- and post perativ pure t e audiograms.

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History This 36-year-old airline pilot noted the onset of fullness and tinnitus in his left ear seven months prior to being seen There was no vertigo unsteadiness or hearing loss.

Examination Neurologic examination was normal Eighth nerve (Fig 8) SRT 5 dB PB 96% Békésy Type II SISI 0%-2000 15%-4000 Vestibular Electronystagmography was normal Cerebral spinal fluid protein 30 mg %

X ray Temporal bone x rays showed a slight enlargement of the left internal auditory canal The meatus appeared normal

PROCEDURE AND MATERIAL

An account has already been given of both the equipment (Blegvad, 1966) and the procedure (Blegvad & Terkildsen, 1966, 1967). As described by Jerger *et al.* (1959), the SISI test involves a relatively rapid and simple procedure, the test technique is extremely well designed and it offers essential advantages in comparison with Lüscher's test. However, the test only provides information on the ability of the patient to perceive increases in intensity of 1 dB magnitude. As it was of interest to have a more precise evaluation of the influence of contralateral masking on the sensitivity of the ear to changes in intensity, the investigation was extended as mentioned, and as far as possible the psychometric function was evaluated both without and with masking in each case. Series of 20 increments of constant size within each single test-run, but varying from series to series, were presented alternatively with 0 dB masking and with 80 dB broad-band noise delivered to the contralateral ear. In the original design of the apparatus, the magnitude of the increments could be varied in steps of 0.5 dB from 5.0 dB down to 0.5 dB. It became clear at a very early stage, however, that some patients were able to hear changes in intensity even as small as 0.5 dB without any particular difficulty, so an adjustment of 0.3 dB was added to the attenuator. During the investigations, it was found that in a few cases even these increments were not sufficiently difficult to detect in order to permit a study of the masking effect, since scores were almost 100% both with and without masking. However, it was considered that the experimental equipment described did not allow an increment less than the one mentioned to be calibrated with reasonable accuracy. The test signal and the masking noise were delivered by insert receivers. The intensity of the continuous tone delivered was 20 dB above the threshold for the affected ear. This threshold was determined without masking, and even though masking noise might in some cases be expected to change the threshold (on account of middle-ear muscle contraction and central masking), the level of the test tone was maintained constant. For the inclusion of a patient in the material, it was not a demand that the hearing of the good ear was within normal limits. Whatever the threshold in the better ear, the masking was delivered at an intensity of 60 dB SPL. This intensity was chosen as being representative of clinical masking.

The material consists of 32 patients suffering from a predominantly unilateral hearing loss of perceptive type. Their ages varied from 25 to .4 years (mean 51 years). With regard to diagnoses, the distribution was as follows: Menière's disease 24, sensorineural unknown 4, sudden onset of loss 2, Lermoyez disease 1, presumably congenital loss 1. The diagnosis of Menière's disease presupposed the symptoms of hearing loss, tinnitus and disturb-

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DIFFERENTIAL INTENSITY SENSITIVITY AND CLINICAL MASKING

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Patients with unilateral perceptive hearing loss have been examined to determine whether masking of the good ear influences the difference limen of intensity. The measurements were carried out with the aid of the SISI procedure using increments of different magnitudes, until the psychometric function was determined both without and with masking (80 dB SPL). The level of the test tone was maintained constant (20 dB above the threshold determined without masking). At 1000 and 4000 cps, the contralateral noise resulted in a significant improvement in the intensity discrimination, while no change occurred at 250 cps. With a few exceptions, the masking had no significance for the result of the topognostic SISI test using 1 dB increments.

Two decades have elapsed since measurement of the difference limen of intensity (DLI) was introduced as a test in diagnostic audiometry (Lüscher & Zwislocki, 1948). As mentioned in a previous paper (Blegvad, 1966) a number of investigations have been made on the diagnostic value of Luscher's test. Especially since the description of the SISI test by Jerger *et al.* (1959) the investigation of the sensitivity of the ear to changes in intensity has found wide application in the topical diagnosis of hearing loss. Several studies are also now available on the reliability of the SISI test and on a range of variables which are of significance for the results of the test. A review of these studies has been provided by Harford (1967). Difference limen measurements have been employed both in the case of unilateral and bilateral hearing loss. Although not always reported in the literature, it is probable that masking of the good ear has been a regular procedure in the examination of patients with unilateral hearing loss. As reported in a preliminary communication (Blegvad & Terkildsen, 1966) we have observed that in some patients with unilateral perceptive hearing loss, the results of the investigation are influenced by the use of masking noise. As far as we are aware, this factor has not been taken into consideration previously, and the aim of the present investigations is to evaluate this problem in greater detail.

This work was supported by a grant from Statens almindelige Videnskabsfond.

be carried out.[1] Finally and most important, the investigation was so time-consuming that in ambulatory patients it could be carried out at only one frequency. In general, between 1 hour and 1 1/2 hours was devoted to each frequency. The total of complete investigations at each separate frequency was as follows: 250 cps, $n = 21$; 1000 cps, $n = 23$; 4000 cps, $n = 20$.

RESULTS

The results were analysed in the same way as in the investigation of the influence of contralateral masking on the SISI test in normal hearing subjects. In each psychometric function, four values of intensity increment were chosen which had given the nearest scores around 50%. In some cases, only three values could be used, and in a few cases only two values, as even the least increment had given a score near 50%. In these cases the 0% point of the curve was calculated by linear extrapolation. The slope of the psychometric functions and the 50% point of the functions were determined by means of the area method. The slope was calculated as the increase in score (in %) for an increase in increment magnitude of 1 decibel. The 50% point indicates the increment magnitude at which on the average the subject will reply on half the number of occasions, and is usually taken to indicate the DLI. The *t*-test was used to determine whether the mean difference between the values obtained without and with masking differed significantly from 0.

Table 1 gives the results for the slope of the functions. As stated, masking gave no significant shift in slope. Table 2 shows the results for the 50% point. Both at 1000 and 4000 cps, the masking resulted in a significant shift towards a lower value. The SISI scores for 1 dB increments were classified in the usual three categories: 0–15% negative, 20–55% questionable, 60–100% positive. The distribution of the results, without and with contralateral masking (the latter shown in italics) was as follows: 250 cps: negative 15, *14*; questionable 5, *6*; positive 1, *1*. 1000 cps: negative 7, *5*; questionable 4, *6*; positive 12, *12*. 4000 cps: negative 9, *6*; questionable 5, *7*; positive

TABLE 1. *Mean slope of psychometric functions for intensity variations in quiet and with masking of the good ear. Results from patients with unilateral perceptive deafness*

Slope: Mean of individual slopes determined with SISI method expressed in % per 1 dB. The changes with masking were not significant at the 5% level.

Masking level (dB SPL)	250 cps ($n = 21$)		1000 cps ($n = 23$)		4000 cps ($n = 20$)	
	Slope	Change	Slope	Change	Slope	Change
0	66.8		101.9		97.3	
60	68.6	1.9	9 .6	−2.5	104.8	7.1

1 In the course of the investigations we encountered this problem in four patients and only at 1000 cps. It is evident that the omission of these patients with particularly acute differential sensitivity influences our mean DLI at this frequency as well as the distribution of SISI scores.

ance of equilibrium, whereas it was not regarded as necessary criteria that the patient should suffer from vertigo in acute attacks or that his acoustic symptoms should fluctuate in step with any possible attack. No patient was included in the material if the result of Fowler's binaural balance test and/or Metz (1952) test was negative. With regard to the audiometric configuration of the hearing loss, the distribution was as follows (classified according to Johnson & House 1964) High tone 6 low tone 7 flat loss 17 trough shape 2.

The investigations were made at 250 1000 and 4000 cps The threshold of the frequencies investigated was within the limits 35–75 dB (*re* ISO 1964 norm) The sequence in which the frequencies were investigated varied, just as the different increment magnitudes were presented in varying order With regard to the reliability of the SISI test Jerger *et al* (1959) quote the results of investigations on a single patient who was examined 8 times over a period of 10 months by three different investigators. At 1000 cps the scores varied from 0 to 5% at 4000 cps the maximum variations were from 65 to 95% This stability was characterized as typical Jerger subsequently (1962) investigated test–re-test reliability by requesting 27 patients with perceptive hearing loss to attend for re-examination The greatest test–retest difference (12.3%) was found at 4000 cps. The coefficient of reliability (r) was found to be low at 250 cps (0 10) but the determination is claimed to have suffered from a quite restricted range as large SISI scores are rare at this frequency At 1000 and 4000 cps there was reasonable consistency ($r = 0\ 72$ and 0.88 respectively) It is our experience that even within the same sitting the results of the investigations can show considerable variations, variations which can be ascribed neither to training nor to mental fatigue With the same stimulus, the score can even fluctuate between positive and negative Since in some patients a contralateral masking effect seemed to be present in an extremely discreet form, a single measurement was not considered sufficiently positive evaluation Determining the psychometric function both with and without masking, the measurement was repeated using those increments which had given scores around 50% i.e those variations in intensity which were at the boundary of audibility In most cases, a single re-test was not considered sufficient and test runs with those increments which represented the steep portion of the psychometric curve were repeated 2–3 times. On the average, 4 repeat test runs were carried out at 250 cps, 5 at 1000 cps and 6 at 4000 cps At 250 cps, the psychometric function was generally less steep than at higher frequencies, and the course of the curve was determined at a greater number of measuring points. For this reason fewer re tests were made at this frequency despite its low coefficient of reliability The mean values were employed in the statistical analysis. Only a proportion of the patients could be examined at all three frequencies In a number of cases the threshold lay outside the limits chosen and in a few cases the sensitivity to the variations in intensity was so great that as stated above the investigation could not

be carried out.[1] Finally and most important, the investigation was so time-consuming that in ambulatory patients it could be carried out at only one frequency In general, between 1 hour and 1 ½ hours was devoted to each frequency The total of complete investigations at each separate frequency was as follows 250 cps, $n = 21$ 1000 cps, $n = 23$ 4000 cps, $n = 20$

RESULTS

The results were analysed in the same way as in the investigation of the influence of contralateral masking on the SISI test in normal hearing subjects. In each psychometric function, four values of intensity increment were chosen which had given the nearest scores around 50 %. In some cases, only three values could be used, and in a few cases only two values, as even the least increment had given a score near 50 % In these cases the 0 % point of the curve was calculated by linear extrapolation The slope of the psychometric functions and the 50 % point of the functions were determined by means of the area method. The slope was calculated as the increase in score (in %) for an increase in increment magnitude of 1 decibel The 50 % point indicates the increment magnitude at which on the average the subject will reply on half the number of occasions, and is usually taken to indicate the DLI. The *t*-test was used to determine whether the mean difference between the values obtained without and with masking differed significantly from 0

Table 1 gives the results for the slope of the functions. As stated, masking ga e no significant shift in slope Table 2 shows the results for the 50 % point. Both at 1000 and 4000 cps, the masking resulted in a significant shift towards a lower value. The SISI scores for 1 dB increments were classified in the usual three categories *0–15 %* negative *20–5 %* questionable *60–100 %* positi e. The distribution of the results, without and with contra lateral masking (the latter shown in italics) was as follows 250 cps nega tive 1a, *14* questionable 3 *6* positive 1 *1* 1000 cps negative 7 *5* questionable 4, *6* positive 12, *12* 4000 cps negative 9 *7* questionable 3 *7* positive

TABLE 1 *Mean slope of psychometric functions for intensity variations in quiet and with masking of the good ear Results from patients with unilateral perceptive deafness*

Slope M an of individual lopes determined with SISI method expressed i % per 1 dB The ha ges with masking were not significa t at the 5% lev l.

Masking level (dB SPL)	250 cps (n 21)		1000 cps (= 23)		4000 cps (20	
	Slope	Change	Slope	Change	Slope	Change
0	66.8		101 0		97.5	
60	68 8	1 9	97 6	3.5	104 6	7 1

In the course of the i estig tions w encountered thi problem i fou pati t ed only at 4000 cps. It is evide t that the mixalo f these patients with particularly acute differential sensiti ity influences our mea DLI t thi frequency as w ll as the distribution of SISI scores.

TABLE 2 *Mean DLI (dB) of patients with unilateral perceptive deafness in quiet and with masking of the good ear*

DLI: Averaged 50% points of psychometric functions for intensity variati ns as determined with SISI method. Asterisk indicate significance le el of the changes caused by masking (and for $p < 1\%$ and 0 1% respectively)

Masking level (dB SPL)	250 cps ($n = 21$) DLI	Change	1000 cps ($n = 23$) DLI	Change	4000 cps ($n = 20$) DLI	Change
0	1 77		1 26		1.35	
80	1 68	−0.03	1 08	−0.18	1.13	−0.22

6 6 Thus, the masking influenced to topognostic result of the current clinical test in only a very few cases. Even though the mean change in DLI as a result of the contralateral masking was only slight numerically the change in score was at times striking, as shown below

At 1000 cps, the score in patient K. K. in four test-runs with 0.5 dB increments was *5 35 40* and *35%* when the contralateral ear was masked without masking, the values in all four cases were 0% At the same frequency the score for Y H. with 1 dB increments and masking was *65 0 50%* while without masking it was 0% on each occasion H K. scored 20 10 20% for 1 5 dB without masking with masking, *80 100 95%* With the same stimulus, K. O H scored without and with masking 5, 5, 15% and *65 45 45%* For a 2 dB stimulus, the corresponding results in this patient were 35 50% and *65 80%*

At 4000 cps, E. S. in five test runs without masking responded to 45 10 35, 50 and 30% of the 0 5 dB stimuli presented with masking to *80 40 75 80* and *60%* With 1 dB stimuli, D P at the same frequencies scored 5, 5, 20% against *50 55 60%* K. S. scored 45 30 45, 55% against *90 95 90 95%* At 1.5 dB in the latter patient, the results were 20 5 35% without masking and *55 45 70%* with contralateral masking. Finally in E. B. without masking the score for 2.0 dB was 15, 25, 30 10% against *65 95 95 80%* with 80 dB masking.

DISCUSSION

The investigations have shown that the difference limen of intensity in patients with unilateral perceptive hearing loss is improved significantly at 1000 and 4000 cps, when the good ear is masked. No change occurs at 250 cps Even though there may have been crosshearing in some of the investigations without masking, this cannot explain the effect of the masking The results for 250 cps tell against the possibility that middle-ear muscle contraction may play a significant role In our investigations of the influence of contralateral masking on the SISI test in normal hearing subjects, we found that the masking noise improved the intensity discrimination at 1000 and 4000 cps, while a significant impairment in discrimination took place at 250 cps. It would seem reasonable to explain the difference found between normal and pathological ears at 250 cps as being due to the difference in

stimulation intensity in both series of investigations the test was performed at 20 dB above the individual threshold, so that the absolute intensity at which the cochlea was stimulated was greater for the pathological group Owens (1965) has pointed out that high SISI scores in cochlear hearing loss can only be expected provided the hearing loss is greater than 30 dB (*re* ASA 1951 standard) Swisher *et al* (1966) have shown that in sensorineural deafness SISI scores (determined 20 dB above the subjective threshold) increase systematically with increasing HL (hearing level the physical intensity of a tone given in relation to the normal threshold for the tone in question) They also found no distinction between the difference limen of intensity in normal hearing subjects and in patients with perceptive loss of hearing, when the basis for comparison was the HL at which the measurement was made These results are in accordance with earlier experience using the Lüscher test In Lüscher's department, while measurements were originally made 40 dB above the patient s threshold (Lüscher & Zwislocki 1948) the procedure subsequently followed (cf for example, Lüscher 1955) was to make the determination at a fixed intensity (80 dB HL) At this intensity the difference limen of intensity in patients with a cochlear type hearing loss (Lüscher in hearing loss with complete recruitment) is said to be equal to that of normal hearing subjects. The observations suggest that the sound pressure level entering the cochlea is decisive for the magnitude of the difference limen. Our results show that the relationships involving the difference limen are more complicated than the above mentioned investigations might suggest At 1000 and 4000 cps, a reduction in DLI was found on noise stimulation of the contralateral ear although the intensity of the test tone was maintained constant all the time and in normal hearing subjects we found contralateral masking capable of eliciting positive SISI scores at 20 dB hearing level (4000 cps) When the results were plotted for the present material (admittedly limited) no striking difference was found between the masking effect at moderate hearing loss and at severe hearing loss (>60 dB) but the relationship between the intensity of the test tone and the masking undoubtedly deserves further study

It has long been known that masking of one ear can change the threshold for pure tones in the other ear According to Lidén *et al* (1959) this effect, usually designated the central masking effect, can amount to 15 dB, even in patients with inactive middle-ear muscles. According to these authors the change is usually of minor importance in clinical audiometry Ward (1963) has stated with regard to clinical masking, perhaps central effects and reflex attenuation will usually be the second-order effects they have been thought to be Our investigation have shown that what is affected is not merely the threshold measured by standard audiometry also the results of Békésy audiometry (Blegvad, 1968) and the difference limen of intensity are affected by contralateral masking Our studies suggest that stimulation of the non-test ear causes changes in the audiological tests which are of both theoretical and practical clinical significance.

TABLE 2 *Mean DLI (dB) of patients with unilateral perceptive deafness in quiet and with masking of the good ear*

DLI: Averaged 50% points of psychometric functions to intensity variations as determined with SISI method. Asterisk indicate significance level of the changes caused by masking (and for $p<1\%$ and 0 1% respectively)

Masking level (dB SPL)	250 cps ($n=21$) DLI	Change	1000 cps ($n=23$) DLI	Change	4000 cps ($n=20$) DLI	Change
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DISCUSSION

The investigations have shown that the difference limen of intensity in patients with unilateral perceptive hearing loss is improved significantly at 1000 and 4000 cps, when the good ear is masked No change occurs at 250 cps Even though there may have been crosshearing in some of the investigations without masking this cannot explain the effect of the masking The results for 250 cps tell against the possibility that middle-ear muscle contraction may play a significant role In our investigations of the influence of contralateral masking on the SISI test in normal hearing subjects, we found that the masking noise improved the intensity discrimination at 1000 and 4000 cps, while a significant impairment in discrimination took place at 250 cps. It would seem reasonable to explain the difference found between normal and pathological ears at 250 cps as being due to the difference in

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CLINICAL APPLICATION OF INTENSITY DIFFERENCE LIMEN

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SISI tests were administered at various sound pressure le els in normal and pathologic subjects. When the inner ear receives an audible ignal of 60 dB SPL or greater a positive SISI score will occur in both normal and sensorineural hearing losses except *in those with abnormal dapta*tion. A egati e SISI score in the absence of a conductive barrier occurs only in abnormally adapting ears *and is probably indicative of supra*threshold adaptation. Our data ndicate that recruiting ears and normal ears perceive *intensity increments f equal size l equivalent* SL. In abnormally dapting ears, as Békésy separation i creases, the increment *tends to become larger than normal.* Continuous tone Békésy thresh ld measured at the bottom of the first spike increase directly with starting intensity *and i ersely* with attenuation rate Vari ti ns of the SISI test and Békésy audiometry are proposed.

The intensity difference limen (DLI) *has been measured by many* methods. The Lüscher & Zwislocki (1948, 1949) and Denes & Naunton (1950) *clinical tests ha e been replaced by the widely used SISI test (Jer* ger *et al* 1959) The amplitude of Békésy tracings has also been considered an indication of the DLI (Békésy 1947) *Closely related* to tests of the DL are the phenomena of recruitment, defined as an abnormal growth of loud ness, and adaptation, described as a reduction in loudness during stim ulation. Recruitment is classically measured by the alternate binaural loud nes balance test (Fowler 1928) or the monaural loudness balance test (Reger 1936) Adaptation may be measured at threshold by the tone decay test (Schubert, 1944 Carhart, 1957) and by Békésy audiometry Threshold drift and wide separation between pulsed and steady tone Békésy tracings are examples of threshold adaptation. Suprathreshold adaptation may be measured by a simultaneous loudness balance procedure described by Hood (1950) and by a delayed loudness balance test reported by Wood (1930)

PART I

The size of the difference limen for int nsity in normal ears varies among subjects and depends on the method of testing. Nine years after the intro-

Presented at the combined meeting of Sections on Otolary gology College of Physicians of Philadelphia, and the New York Academy of Medicine with the Philadelphi Laryngological Society at Philadelphia, Pen syl ania on March 20, 1968

ZUSAMMENFASSUNG

Man hat bei Patienten mit einseitiger perzeptiver Schwerhörigkeit untersucht, ob die Maskierung des guten Ohres Einfluss auf die Intensitatsunterschiedsschwelle haben werde. Die Messungen wurde mittels der SISI Prozedur mit Intensitätsänderungen verschiedener Grösse unternommen bis die psychometrische Funktion teils ohne teils mit Maskierung (80 dB SPL) festgestellt wurde Das Niveau des Test Tons wurde konstant gehalten (20 dB über die Schwelle wie ohne Maskierung gemessen) Bei 1000 und 4000 Hz verursachte das kontrolaterale Geräusch eine signifikante Besserung der Intensitätsdiskrimination, bei 250 Hz geschah keine Änderung. Mit wenigen Ausnahmen war die Maskierung ohne Bedeutung für das Ergebnis der topognostischen SISI Prüfung mit 1 dB Intensitätsänderungen.

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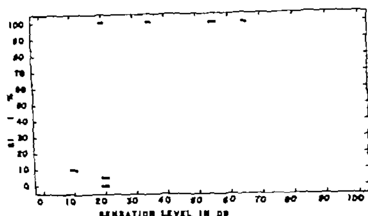


Fig. 3. SISI scores of the same 17 cochlear lesions with thresholds lower than 60 dB SPL plotted on the basis of sensation level.

to near 100% above 50 dB at all frequencies tested. In naive normal subjects, this change occurs at 60 dB (Fig. 1). In two groups clinically diagnosed as having either Menière's syndrome or abrupt drop hearing loss, the change also occurs at 60 dB as in naive normals (Young & Harbert, 1967). It is universally accepted that these cases have cochlear lesions, yet they do not differ from normal ears without hearing loss when intensity is plotted in SPL. When the same data of the Menière's group is plotted as a function of SL, all of these cases showed SISI scores between 80 and 100% at sensation levels both below and above 20 dB. Presbycusis and congenital sensorineural hearing loss show the same abrupt change at 60 dB as normals and admittedly cochlear lesions (Young & Harbert, 1967). Because there were few subjects with hearing levels below 60 dB SPL in this series, another group of cochlear lesions with minimal hearing loss was tested. Fig. 2 plotted in terms of SPL shows that this group of 17 behaved similar to the



Fig. 4. SISI scores of 17 cases of conduction hearing losses as function of SPL without subtracting the conduction barrier.

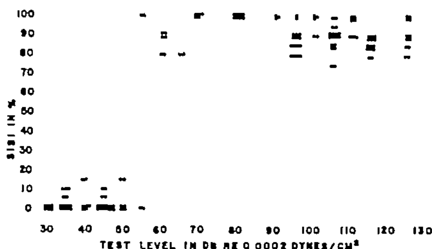


FIG. 1 SISI scores of 33 naive normal listeners tested at various frequencies.

duction of the SISI test, investigators and clinicians are still not in agreement on either the best size of the increment or the significance of the test.

The SISI test records the percentage of 1 dB increments heard out of 20 trials. The increments are superimposed on a continuous tone delivered at 20 dB sensation level (SL) Normal ears and those with a significant conductive hearing loss or abnormal adaptation fail to hear most, if not all of the 20 increments. Ability to hear a high percentage of the presentations is considered indicative of a "cochlear" lesion (Yantis & Decker 1964 Owens, 1965)

By employing sensation level the inner ear is subjected to variable intensities depending on the degree and type of hearing loss If it can be shown that a pathologic ear behaves as a normal ear when subjected to signals at equivalent intensities, the diagnostic significance of the test is in doubt

When percentage score is plotted against SPL in trained normal listeners, there is a sharp change from near zero % at intensities below 50 dB SPL

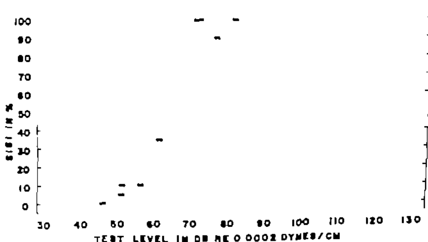


FIG. 2. SISI scores of 17 cochlear lesions with minimal hearing losses (thresholds less than 60 dB SPL at frequencies from 250 Hz through 8000 Hz) as a function of SPL.

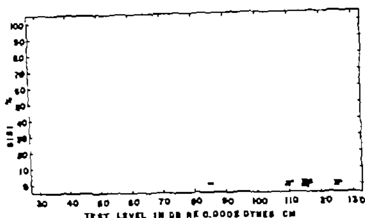


FIG. 6. SISI scores of 13 abnormally adapting ears tested at intensities above the established Békésy continuous tone threshold.

The SISI test is usually administered with a high signal to noise ratio. Tonndorf *et al* (195) and Sherrick (1950) have shown that as the S/N ratio increases the DLI becomes smaller and that sensation level is not important. These investigators sought the size of the DL by varying the S/N ratio and concluded that for a given S/N ratio, the DL was constant and independent of the SL. In this study the increment was constant and the per cent score was plotted as a function of S/N ratio. If noise is deliberately added to a signal at 60, 80, and 100 dB SPL, there is a sudden change in the SISI score for a 1 dB increment when the S/N ratio is reduced to +5 dB or below. This change from near 100% to near 0% is as dramatic as the change from 30–60 dB in the absence of noise (Fig. 7). When the increment is increased above 1 dB, detectability in the presence of noise is enhanced. For a 2 dB increment the signal to noise ratio is −5 dB, for a 5 dB increment it is −15 dB.

In a study (Weiss *et al* 1967) on the minimum size of the increment that is recognizable in normal and pathological ears, the following findings are of interest

1 In 63 naive normal ears, the size of the increment varied between 0.5 and 1.5 dB when a 230 msec increment with a rise and fall time of 25 msec was presented near the end of a 5 second, 80 dB SPL, pure tone signal.

2 In 41 ears with recruitment the intensity increment fell within this norm l range. Our data also did not sustain the notion that recruiting ears can detect smaller increments than normal ears at equivalent SPL by this method.

3 In abnormally adapting ears, increment data suggested that as separation between continuous and interrupted Békésy tracings increases, the increment tends to become larger than normal.

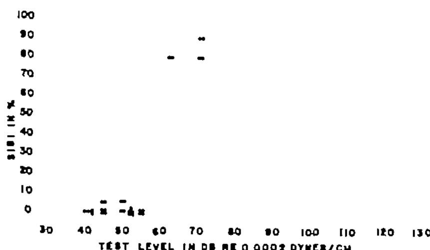


FIG. 5 SISI scores of the same 17 conductively deafened ears as a function of SPL after the conductive barrier was subtracted from the testing levels.

naive normal group Again when the same data are plotted as a function of SL, it is apparent that more than half show low SISI scores at 20 dB SL (Fig 3) All of the positive scores were in subjects receiving the signal at 55–65 dB SPL. Low SISI scores occurred when subjects received the signal at 55 dB SPL or below This is in accord with the findings of Harford (1965) that low SISI scores accompany mild losses in some cases of cochlear pathology This emphasizes that SPL rather than SL is the important parameter in determining the score value.

When percentage scores in conductively deafened ears are plotted on the basis of SPL, there are a large number of negative scores even at high intensities (Fig 4) When these same data are plotted after subtracting the conductive barrier these ears also show an abrupt change in SISI scores at 60 dB as in all of the above (Fig 5) Only abnormally adapting ears show negative SISI scores at SPL both above and below 60 dB (Fig 6)

These findings indicate that a positive SISI score in a naive listener *means the inner ear is receiving a signal at an intensity of 60 dB SPL or above and is behaving as a normal ear at this intensity* Since trained normal listeners notice this change at 50 dB SPL, it appears likely that subjects who undergo repeated testing or are acute observers may also respond with high scores at this level and above This is in keeping with Riesz s finding (1928) that in normal ears the differential sensitivity for frequencies 32–8192 Hz varied between 0 2 and 0.5 dB from 60 dB to 130 dB SPL by his method of testing Swisher (1966) and Swisher *et al* (1966) also showed that normal and non adapting sensorineural impaired ears discriminated a signal of 1 dB or less equally well at equivalent SPL.

If the SISI score is low (negative) when the inner ear receives a signal at 60 dB SPL or above, it probably indicates an abnormally adapting ear and this behavior can be equated with abnormal tone decay significant separation between pulsed and steady tone Békésy tracings, etc

approached alternately from no sound to sound and sound to no sound, the single number representing threshold was considered to be a line connecting the midpoints of spikes. On the basis of thresholds for various attenuation rates and relation to conventional audiometry we consider the bottoms of spikes the best single measure of threshold (Harbert & Young, 1962, 1966) If the threshold is approached from sound to no sound the threshold of inaudibility is invariably better than the threshold of audibility determined by proceeding from no sound to sound. This differene represents the amplitude of Békésy spikes. For some reason, if the ear is hearing a decreasing signal it is able to continue hearing in an area where it never hears when the sound is increasing from an inaudible level The more rapid the rate of change, the better the threshold of inaudibility as measured by the amplitude of spikes. If the amplitude is known for a given attenuation rate, the amplitude for other attenuation rates can be predicted Doubling the attenuation rate increases the amplitude by about 62% (Harbert & Young, 1966) Reduced amplitude therefore represents a defect in the ability to continue to hear a sound decreasing from threshold. We consider this a manifestation of rapid adaptation which takes place in milliseconds.

The disorder which causes reduced amplitude of continuous tone Békésy tracing does not cause a corresponding reduction of pulsed tone amplitude There appears to be no close correlation between amplitude width and recruitment as measured by the alternate binaural loudness balance test. The concept of threshold recruitment implies that the reduced amplitude represents a "sharper" threshold than normal. In other words, the pathological ear performs better than a normal one.

For pulsed tones, the threshold of the bottom of the first spike is the same whether threshold is approached from below or above except in certain markedly adapting ears. For continuous tones, there is a threshold increase of 1.5 dB for every 20 dB increase of suprathreshold starting intensity in normal ears. There is a similar 1.5 dB increase in threshold for each halving of the attenuation rate in both normal ears and those with cochlear lesions. For a group of cochlear lesions each 20 dB increase in suprathreshold starting intensity causes a mean elevation of threshold of about 5 dB. In markedly adapting ears, the effect may be much more Frequency does not seem t be a factor (Harbert & Young, 1968)

These findings may be the basis for another clinical test for abnormal adaptation.

1 The threshold for a fixed frequency continuous tone is approached three times from below and the mean recorded

2. Th threshold is similarly approached from the limit f the machine and it mean recorded.

3. Th difference between these would be a measure of adaptation to be compared with the normal amount.

4 In ears with marked abnormal adaptation there may be a similar but lesser effect for pulsed times.

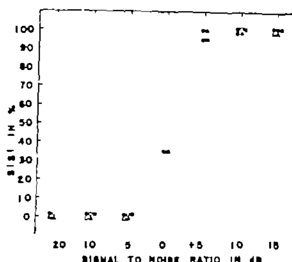


FIG. 7 SISI scores for 1000 Hz at 100 dB SPL in five trained normal listeners as f nc tion of S/N ratio.

In order to minimize adaptation to the carrier signal in the SISI test, it is suggested that the test be modified as follows

1 After a practice period to ensure recognition of the increment and understanding of the test, interrupt the signal after each presentation by briefly (1 second) depressing the interrupter switch Record score for each presentation The selector switch to eliminate the increment should be randomly activated after several positive responses have been obtained to eliminate a false positive response

2 The increment should be set at 1.5 dB and the signal presented at 70 dB re ISO (1964) for frequencies 500 to 8000 Hz and 55 dB re ISO for 250 Hz. When hearing loss approaches these levels, the signal should be increased to an intensity at least 10 dB above threshold. The conductive barrier in dB should also be added In abnormally adapting ears, the test level must be above the stabilized Békésy threshold for valid results.

3 A positive score means that the ear is responding as a normal ear If a negative score is obtained the size of the increment may be increased until it is heard to measure the degree of impairment of the DLI

PART II

The Békésy audiometer is a device to record the threshold of a tone that is changing in intensity at a constant rate usually by a method of limits technique Instructions are to press a button as soon as the tone is heard and to keep it depressed until it disappears While the button is depressed the signal decreases On the tracing, every rising limb represents a period of hearing and every descending limb a period of no hearing This has been described as "variability about the threshold" (Hirsh *et al* 1954) but this variability is not random Because the threshold was considered to be

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CONCLUSIONS

1 A positive SISI score when the inner ear perceives a signal of 60 dB SPL or more, probably has no significance since it occurs in both normal ears and sensorineural deafened ears with a wide variety of diagnoses.

2 A negative SISI score when the inner ear receives an audible stimulus at 60 dB SPL and above, is an indication of abnormal *intensity discrimination* and is probably indicative of suprathreshold adaptation.

3 Reduced amplitude of Békésy continuous tone tracings probably is a measure of rapid adaptation.

4 Variations of SISI and Békésy tests are proposed.

ZUSAMMENFASSUNG

An normalhörenden Personen und pathologischen Fällen wurde die Änderung der Quote des SISI Tests mit wachsendem Schalldruckpegel aufgenommen Wird dem Ohr ein für den Patienten hörbares Signal mit einem Schalldruckpegel von 60 dB oder mehr zugeleitet, so ist die SISI Test-Quote sowohl bei normalhörenden Personen wie bei neurogenen (sensorineural) Hörverlustfällen positiv jedoch unter Ausnahme der neurogenen Fälle mit abnormaler Adaptation Ein negativer Ausfall des SISI Tests findet sich sofern keine zusätzliche Leitungsfehlkomponente vorliegt, nur in Fällen mit abnormaler Adaptation Aus den vorliegenden Daten ist des weiteren zu entnehmen dass bei gleichem Schalldruckpegel die gerade wahrnehmbare Intensitätszunahme für Ohren mit Recruitment und für normalhörende Ohren von gleicher Grösse ist. In Fällen mit abnormaler Adaptation dagegen bei denen die Békésy Audiogramme mit kontinuierlicher und pulsierender Tonfolge in zunehmendem Masse divergieren fallen die gerade wahrnehmbaren Intensitätsänderungen eher grösser als normal aus. Weiterhin wurde die Abhängigkeit des Beginns der Schwellenzeichnung der Békésy Aufnahme mit kontinuierlicher Tongabe (bei konstanter Frequenz) von der Einsatzintensität der Tongabe und der Geschwindigkeit der automatischen Intensitätsänderung untersucht. Im Zusammenhang damit werden gewisse Modifikationen in der Durchführung des SISI Tests und hinsichtlich der Einsatzintensität der Tongabe bei der Békésy Audiogrammaufnahme vorgeschlagen

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Nerve innervation of the bronchial tract, such as excitatory fibers from the vagus and inhibitory fibers from the sympathetic to the bronchial muscle, is mainly concluded on the basis of physiological evidence. Although some attempts (Larsell, 1922 Honjin, 1950 Fisher 1965) were made to observe the nerve innervation of the respiratory tract by staining with silver or methylene blue, the adrenergic nerves could not be differentiated from other types of nerves. Concerning the study of the distribution of the adrenergic nerves by histochemical demonstration of the monoamine oxidase, Fujiwara *et al.* (1966) showed that this enzyme tended to distribute to the effector cells.

The fluorescent histochemical demonstration of the endogenous n radrenaline developed by Falck (1962) seemed to be the best histological device available to study the adrenergic innervati n of the ti sues. According to the simplified modification of Falck s method, Terayama & Soda (1967) demonstrated the noradrenaline-fluorescent fibers only in the vessels of the submucous and muscular layers of the esophagus. Dahlstrom *et al* (1966) observed the specific flu rescent fibers innervating the bronchial muscles in cats and suggested that some part of the innervati n derived from the pathogenesis of the bronchiectasia.

In the present experiments the presence of the specific fluorescences of noradrenaline produced by the exposure of the freeze-dried tissue sec tions to formaldehyde in the esophagus and bronchial tract of the rabbit were histochemically observed.

MATERIAL AND METHODS

Twenty male albino rabbits, each weighing about 2 kg, were used The animals w re sacrificed by the exsanguination from the cut ends of both common carotid arteries, and immediat ly thereafter the whole length of the esophagu and both lungs with the trachea were extirpated The esophagus was divided into three parts, the upper (cervical) middle (thorac l) and low r (thoraco-lumbal) thirds. The respiratory tract was also divided int three parts, the cervical trachea, tracheal bifurcatio, and lung. Along with the rabbits, some guinea-pig and rats were also used.

The fluorescent histochemical procedures of the esophagus and respirat ry tract w re performed according to the original method of Falck (1962)

The freeze-dried p eparation were treated with f rmaldehyde gas at 80 C f 1 hr then infiltrated *in vacuo* with paraffin t 60 C for 10 min. Sect on (4–8 μ) w re placed on non-fluorescent slides nd deparaffinized by car ful addition f xylen The microscopical observation of the tissue section w perf rmed by means of a Zeiss fluorescence microscope eq ipped with an HBO 200 high p essure mercury lamp, a Schott BG 12 (3 mm exc tation filter dark field condenso nd a Schott OG 4 filt r (1 mm) in the tube.

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THE ADRENERGIC INNERVATION IN THE ESOPHAGUS AND RESPIRATORY TRACT OF THE RABBIT

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The mode of innervation of the adrenergic nerve fibers in the esophagus and respiratory tract of the rabbit was observed fluoro-histochemically The noradrenaline fluorescent fibers in the esophagus were found to distribute in the submucosa lamina muscularis mucosa muscle layers, and around the myenteric ganglion cell. The dense distribution of the fibers around the myenteric plexus, arteries, and arterioles showed the net structures with varicose terminals, indicating active regulation of the structures. The fluorescent fibers in the trachea were found to distribute in the submucosa and muscular layers by penetrating the fibro-elastic layer and membraneous portion of the submucosa The distribution of the fluorescent fibers in the lung was relatively abundant around the pulmonary arteries, but the smooth muscle of the bronchiole exhibited a few fluorescent fibers, which were completely absent from the alveoles

A peristaltic wave appears in the upper end of the esophagus associated with each swallowing effort This wave consists of an esophageal constriction caused by the contraction of the external bundles of striated muscle at the site of the wave and relaxation beyond The striated muscle is directly innervated by the vagus nerves without the intervention of any local ganglion cells. In the smooth muscle portion of the lower third of the esophagus, however there is an extensive local plexus including nerve cells and their branches similar to the myenteric plexus in other parts of the gastrointestinal tract (Greving 1910) Further in animals such as dogs and rabbits, in which the entire esophagus is made up of external striated muscle high cervical vagotomy results in a permanent inability to swallow Based on the observation that the thoracal and cervical sympathectomy in dogs produced an increased sticking of the food on the mucous membrane of the esophagus, Kure & Okinaka (1956) suggested adrenergic participation with the esophageal motility The longitudinal internal muscularis mucosa of the esophagus is a smooth muscle and it increases in thickness toward the cardia of the stomach In spite of several histological attempts (Kadanoff & Spassowa, 1959 Temesrékasi, 1959 Liven, 1961) no clear-cut evidence to support the regulatory mode of the sympathetic innervation on the esophageal function ha been presented hitherto in available literature



Fig. 2. The middle portion in the esophagus of normal rabbit. The pecific fluorescent fibers (↑) are much more ab ndant in the xternal muscl layer (*EL*) than i the medial (*ML*) ad internal layers. The pecific fl orescent fibers i the ad entitia of an artery (*AT*) are observed bet een the external and medial layers. ×126.

muscle, showed a fairly dense distribution of the specific fluorescent fibers interstitially (Fig. 2). On the other hand, the fluorescent fibers were much less in the circular muscles and especially in the smooth muscle of the lamina muscularis mucosa. Auerbach's ganglion cells, found in the myenteric space between the longitudinal and circular muscle layers, were surrounded by a dense network f fluorescent varicose fibers (Fig. 3). These fibers showed occasionally a continuous connection with the fluorescent fibers penetrating into the external longitudinal and internal circular muscles. Auerbach's ganglion cells were non fluorescent. The arteries with the intermediary caliber observed frequently in the longitudinal muscle and adventitia, also showed dense distribution of the fluorescent fibers, mainly in their ad entitial layers (Fig. 4).

No significant difference in distribution of the specific fluorescent fibers was found between the upper and middle thirds of the esophagus. On the other hand, the fluorescent fibers and Auerbach's nerve cells, with the surrounding dense net of the fluorescent fibers in the lower third of the esophagus, were found to be increased toward the cardia of the stomach together with an increase in thickness of the inner longitudinal muscularis mucosa.

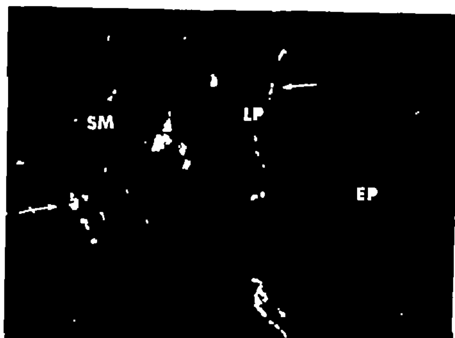


Fig. 1 The upper portl n In th esophagus of a normal rabbit. The pecific fluorescent fibers (↑) in the l mina propria mucosa (*LP*) and the ubmucosa (*SM*) are abundant, but are absent fr m the epith lium (*EP*) ×160

The endogenous noradrenaline in the tissues exhibited a green or yellowish green fluorescence In addition the specific fluorescence of noradrenaline was confirmed by comparison of the adjacent tissue section without treatment with paraformaldehyde with the tissue sections taken from the animals previously treated intraperitoneally with 1 mg/kg of reserpine 18 hours before

RESULTS

Distribution of the adrenergic nerves in the esophagus

The rabbit esophagus consists of the following five layers the mucous epithelium lamina propria mucosa, submucosa, circular and longitudinal muscles, and tunica adventitia. The superficial cornification layer of the epithelium and the stratified squamous epithelium exhibited sometimes a non specific fluorescence However a specific fluorescence of noradrenaline did not appear A considerably dense distribution of the noradrenaline-fluorescent fibers was observed in the lamina propria mucosa and the submucosa As shown in Fig 1 the fluorescent fibers in these layers were abundant especially around the arteries and arterioles. However no ganglion cell similar to the Meisner s cell found in the intestinal tract was observed in the submucosa Also, no specific fluorescent fiber was found around the submucosa glands. The muscular layers exhibited also the presence of the specific fluorescent fibers However the distribution of the fluorescent fibers differed considerably in number according to the respective muscle layers. The longitudinal muscularis externa, though it consisted of a striated



Fig. 5. The cervic l portio f the trachea f normal rabbit. The dense distribution of the pecific fluorescent fibers (↓) is observed the ad ntitia of the rteries (*AT*) in the submucosa (*SM*). The fl orescences i the l m na propria (*LP*) are non-specific. 128.

The numerous granular and yellowish fluorescences present in the lamina propria, similar to those found in the medial and intimal layers of the big artery were non specific and seemed to derive from the elastic fibers. The serous and mucous glands in the lamina propria were not surrounded by the specific fluorescent fibers. The arteries and arterioles frequently observed in the submucosa exhibited the dense distribution of the fluorescent fibers mainly in the adventitia (Fig. 5). In addition, the specif c fluorescent fibers penetrating the fibro-elastic layer and the smooth muscle of the membranous portion of the submucosa were also observed, sometimes in



Fig. 6 The aricose fibers emitti g specific fluorescence in the adventitia of the pulmonary artery. The fibers form typical atenomia ground plexus. 320.

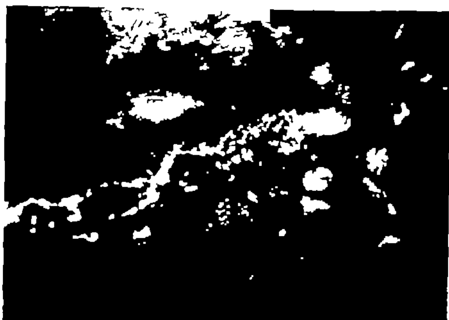


FIG. 3 The lower portion f the esophagus of a normal rabbit Th Au rbach pl xus is found to be surrounded by a dense netw rk of fluorescent v ricose fibers. 320

Distribution of the adrenergic nerves in the respiratory tract

The rabbit trachea consisted histologically of the ciliated pseudostratified columnar epithel lamina propria rich in elastic fibers and poor in the serous and mucous glands, submucosa and cartilage. In the posterior portion of the trachea the glands extended through the muscular layer to the submucosa The epithelial cell did not exhibit the specific fluorescence.



FIG 4 The l we porti n f the sophagu f n rmal rabb t Th pecif fluoresce t fibers re observed the d titia of n art rs (AT) t th t rnal m sel l er (EL) in the medial muscle la (ML d ro d Au rba h pl xus (AP The gangl on cell are n flu rescent 160



Fig. 5. The cervic l portion of the trachea of normal rabbit. The dense distributio of the specific fluorescent f bers ( ) is observed I the ad entiti f the rteries (AT) in the submucosa (SM) The fl orescences I the lamina propria (LP are non-specific 120

The numerous granular and yellowish fluorescences present in the lamina propria, similar to those found in the medial and intimal layers of the big artery were non specific and seemed to derive from the elastic fibers. The serous and mucous glands in the lamina propria were not surrounded by the specific fluorescent fibers. The arteries and arterioles frequently observed in the submucosa exhibited the dense distribution of the fluorescent fibers mainly in the adventitia (Fig. 5) In additi n, the specific fluorescent fibers penetrating the fibro-elastic layer and the mooth muscle of the membranou portion of the ubmucosa were als observed, sometimes in



Fig. 6. The ricose fibers emitti g specific fluorescence i the d entitia of the pul monary rtery The fibers form typical autonomic ground plexus. 320

the portion of the trachea The distribution of the fluorescent fibers did not markedly differ between the cervical trachea and the bifurcatio

Although the pattern of the distribution of the fluorescent fibers in the bronchus was similar to that of the trachea the distribution decreased in density progressively toward the periphery together with a decreased caliber The smooth muscle of the bronchiole exhibited a few fluorescent fibers, which were completely absent from the alveoles However the pulmonary vessels and especially the pulmonary arteries showed the dense distribution of the fluorescent fibers in the adventitia, immediately adjacent to the smooth muscle of the media. These fibers with the varicose structure formed an autonomic ground plexus (Fig 6) as described by Falck (1964) and Norberg (1964) and some of the fibers seemed to invade the media. On the other hand, the pulmonary veins as well as the peribronchial arteries and veins showed decreased distribution of the fluorescent fibers.

DISCUSSION

The greenish yellow fluorescences found in the esophagus and respiratory tract of the rabbit were likely to represent the endogenous noradrenaline in the adrenergic nerve fibers because of the following evidence (1) The fluorescence presented a fiber like structure with a varicose terminal in the fiber course (2) The physiological presence of the endogenous noradrenaline in the esophagus and respiratory tract of the rabbit was also confirmed biochemically by the present authors (1967) The mean levels of the amine in the whole esophagus, trachea and bronchus, and lung were 0 08 0 20 and 0 03 $\mu g/g$ respectively The estimated values seemed to coincide roughly with the distribution of the specific fluorescence found in these tissues

As described above, the vagus nerve has an excitor regulation on the spontaneous and swallowing movement of the esophagus. It was not obvious whether the mechanism of relaxation was derived from the adrenergic nerve or not However Kure & Okinaka (1956) showed the increased sticking of the food on the esophageal mucous membrane in the sympathectomized dog In the esophagus of the rabbit the noradrenaline-fluorescent fibers proved to distribute in the internal medial and external muscle layers, and around the arteries and arterioles in the submucosa and adventitia as well as around the myenteric nerve cell The myenteric plexus in the esophagus of the guinea pig was also confirmed to be distributed with fluorescent fibers, in contrast to the findings of Terayama & Soda (1967) The distribution of the adrenergic nerve fibers around the myenteric plexus in the intestine was first presented by Norberg (1964) fluoro-histochemically Since the myenteric nerve cell confirmed to show a high activity of the intracellular cholinesterase, did not exhibit a specific fluorescence, this cell was concluded to be purely cholinergic in nature However the dense distribution of the fluorescent fibers with the varicose terminals and net structures around the cell indicates the adrenergic innervation, and consequently

the adrenergic regulation, of the cell function. The downward increase in thickness of the longitudinal muscularis mucosa in the esophagus seemed to correlate with the similar downward increase in the number of myenteric nerve cells. Many fluorescent fibers were also found in the lamina propria and submucosa around and apart from the blood vessels.

The mutually antagonistic innervation of the respiratory tract by the autonomic nerves has been established. However no confirming histological study on the adrenergic innervation has been presented. In accordance with the description by Terayama & Soda (1967) the fluorescent adrenergic nerves in the trachea of the guinea pig extended to the submucosal layer by penetrating the fibro-elastic layer and the smooth muscles of the membranous portion and distributed to the smooth muscles and arteries. The fluorescent distribution of the adrenergic nerve fibers to the bronchial muscles was already shown by Dahlstrom *et al* (1966) in the cat. The relatively less developed serous and mucinous glands in the trachea and bronchus of the rabbit did not show the distribution of the adrenergic nerve fibers. The pulmonary artery showed the most dense distribution of the fluorescent fibers, which formed the autonomic ground plexus in the adventitia. Some of the fibers were found to invade the smooth muscle of the media. According to Norberg & Hamberger (1964) and Ohgushi (1967) such distribution of the fluorescent fibers in the smooth muscle of the media was rarely observed in the peripheral artery. The pulmonary veins and peribronchial arteries exhibited a relatively smaller distribution of the fluorescent fibers, but the distribution was significantly more intense than that found in the bronchial muscle. Bruner & Schmidt (1947) and Murao (1966) have shown that the blood flow of the bronchial vessels is increased by adrenergic stimuli and decreased by vagal stimulation. Therefore, adrenergic stimuli can affect more profoundly the bronchial circulation system, enriched by the adrenergic supply than the bronchial muscle poorly supplied by the same nerves.

ZUSAMMENFASSUNG

Es wurde eine fluoreszenzhistochemische Untersuchung über den Innervationsmodus adrenergischer Nervenfasern der Speiseröhre und der Luftwege bei Kaninchen durchgeführt. Die adrenalin-fluoreszierenden Nervenfasern der Speiseröhre liessen sich in der Schleimhaut, Lamina muscularis und in allen drei Muskelschichten sowie um die myenterischen Ganglienzellen nachweisen. Nervenfasern, die sich insbesondere um die myenterischen Nervengeflechte, Arterien und Arteriolen dicht befinden, zeigten die rosenkranzartige Struktur eines Terminalretikulums, wobei eine aktive regulierende Funktion dieser Struktur angedeutet werden konnte. In der Luftröhre war zu erkennen, dass sich die fluoreszierenden Fasern, die elastischen Schichten und Pars membranacea der Submukosa durchsetzend, in der Submukosa und den Muskelschichten verteilten. In der Lunge zeigte sich vor allem um die A. pulmonalis eine relativ reichliche Innervierung obengenannter Fasern, wohingegen sie in der glatten Muskulatur der Bronchiolen kaum zu finden waren und in den Alveolen vollständig fehlten.

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THE BACTERIOLOGY OF THE RESPIRATORY TRACT IN VARIOUS PATHOLOGICAL CONDITIONS

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A total of 16 samples of diseases of the middle ear, nose, paranasal sinuses, and specific and non-specific pulmonary diseases were examined bacteriologically. On the basis of our examinations, we offer the following conclusions. The swab of the ear in acute inflammation of the middle ear has less diagnostic value if the secretion is not pronounced. In these cases we find the bacterial flora sensitive to penicillin. In chronic inflammation the bacterial flora is mixed and antibiotics with the largest spectrum give the best result. The bacterial flora in different pathological conditions of the tracheobronchial tree varies and only a combination of several antibiotics can be successful. The bacteriological examination of the paranasal sinuses must be with clean inflammatory secretion or washing with bouillon, because washing with physiological solution can hide the bacteriological infection with *haemophilus influenzae*. Parallel bacteriological examinations of the nose and paranasal sinuses did not give the same bacteriological findings, which means that the bacteriological finding of the nose cannot be a bacteriological indication of infection of the paranasal sinuses.

During the last 20 years the use of antibiotics has brought about a series of changes in both the clinical and pathological findings in acute and chronic infection. We have witnessed the discovery of a large number of antibiotics with a limited or broad antibacterial activity. However their uncontrolled and widely often schematic, use has sometimes caused their value to be discredited. Furthermore it has caused changes in the bacterial flora as a causative agent of various pathological conditions in the region of the ear, nose, throat, and bronchi. This state of affairs has induced us to study in detail the bacteriological examination in our profession in order to give some directives for antibiotic treatment of infection of the upper and lower respiratory tract. It is certain that infection of the upper respiratory tract can involve the lower respiratory pathways and vice versa, but can do so only if the resistance of the respiratory mucosa is reduced. The flora in the respiratory tract can be of different composition even in areas located as close to each other as the nose and paranasal sinuses. We wanted to give

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special attention to the paranasal sinuses because it has become an established practice in our profession to consider a negative result of puncture as indicative of an absence of infection even if radiological findings are positive This assumption is absolutely false bacteriological examination is of special importance in such cases. While acute otitis in most instances poses no problem with antibiotic treatment because it is generally susceptible to penicillin, chronic otitis and conditions resulting from radical trepanation still remain problems for the otologist We are aware of the fact that the general protective forces of the organism play a certain role in the effectiveness of many antibiotics, in addition to their specific effect—this has to be taken account in the interpretation of the disproportion in the difference of the *in vitro* and *in vivo* effect

A total of 516 samples of diseases of the middle ear nose and maxillary sinus and in specific and non specific diseases of the lung were submitted to bacteriological study

RESULTS

The ear

A total of 138 samples of swabs recovered from the cavum, antrum, or mastoid cells during surgery were examined bacteriologically (see Table 1) Among these 138 examined samples there were 32 which were sterile. Potentially pathogenic flora was found in 86 samples and saprophytic flora in 45 samples. According to the diagnosis, we examined 31 cases of otitis media acuta 82 cases of otitis media chronica, 11 swabs from radical trepanation, 11 samples taken in the course of surgical intervention, and one sample each of otitis bullosa haemorrhagica, blue drum, and otitis externa. In acute otitis media without perforation and in blue drum the discharge

TABLE 1

ST – St rile, PP – pot ntially pathog nic, SA – saprophyte P – pneumococcus, SH – streptococcus haem lyticus, STP – t phylococcu progenes aureus, EC – *Esch richia coli*, PR – proteus, PY – psued monas pyocyanea, KL – Klebsibella, C – candida, STA – staphylococcus lbus, SV – streptococcus iridans, SC – saprophyti cocci D – diphteroid.

						Potentially pathogenic flora							Saprophytic flora			
	No.	ST	PP	SA	P	SH	STP	EC	PR	PY	KL	C	STA	SV	SC	D
1 Otitis med. chr	31	21	4	8	3	1	0	0	0	0	0	0	2	2	2	2
2. Otitis med chr	82	6	72	25	2	4	22	3	25	10	1	2	12	1	8	3
3. Antritis per	8	0	4	4	2	0	1	0	0	1	0	0	3	0	1	0
4 Mastoiditis oper	3	0	3	0	0	0	2	1	0	0	0	0	0	0	0	0
5. St. post oper rad.	11	3	3	6	0	0	2	0	0	1	0	2	3	0	0	0
6. Otitis bul. hem	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7 Blue drum	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8. Otitis externa	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Total	138	32	86	45	6	5	27	4	23	12	2	4	21	3	11	6

was recovered through the ear drum by aspiration with a needle fixed to a syringe.

If we analyse the bacterial flora and the results of bacteriological examinations in acute otitis media, we see that potentially pathogenic flora was found in only 4 out of 31 cases, i.e. pneumococcus in 3 cases and streptococcus haemolyticus in one. Saprophytic bacterial flora either alone or in combination with potentially pathogenic flora was found in 8 cases. Twenty-one samples remained sterile.

The findings of flora and the ratio of potentially pathogenic flora to saprophytic flora differed considerably in cases of chronic otitis media. Potentially pathogenic flora was found in 72 out of 82 examined cases bacteria from the proteus group (25) staphylococcus pyogenes aureus (22) pseudomonas aeruginosa (10) streptococcus haemolyticus (4) and pneumococcus (2) Saprophytic bacterial flora was found in 2ɜ instances, either alone or mixed with pathogenic flora Only 0 swabs in this group remained sterile.

Samples recovered during surgical intervention were all positive out of a total of 11 samples there were 7 positive for potentially pathogenic flora and 4 for saprophytic flora. Among the samples of potentially pathogenic flora there were 3 containing staphylococcus pyogenes aureus, 2 containing pneumococcus, and one each containing *Escherichia coli* and pseudomonas aeruginosa.

In conditions resulting from radical surgical intervention, 3 samples out of a total of 11 swabs were sterile, potentially pathogenic flora was isolated from 3 swabs, and saprophytic flora from 6 Staphylococcus pyogenes aureus was found twice and pseudomonas pyocyanea once in the samples of potentially pathogenic flora.

Samples recovered in blue drum and otitis bullosa haemorrhagica remained sterile saprophytes only were found in otitis externa.

Bronchi

Technique f recovering swab Sterile glass tubes slightly thinner and longer than a bronchoscope served as protection from pollution on the way to the bronchi for the sterile cotton swab fixed to a wire.

A total of 120 samples of bronchial discharge from the same number of patients were examined bacteriologically (see Table 2) Of this number 87 were uffering from pulmonary disease due to mycobacterium of tuberculosis, i.e. 38 with th diagnosis atelectasis, 26 with infiltratio pulm., 16 with adenitis hili pec., 4 with pneumonia caseosa, and 3 with tbc. miliaris pulm. Twenty two of a t tal of 87 samples remained sterile potentially pathogenic flora and 112 saprophytic causative agents were found in 57 Potentially pathogenic flora was isolated as follows streptococcus haemolyticus (22) pneumococcus (16) staphylococcus pyogenes aureus (14) haemophilu influenzae (7) *E. coli* (2) and candida (1) In almost all cases these causati e agents were isolated concurrently with abundant sapro-

TABLE 2

HI = *Haemophilus influenzae* EN = enterococcus, SN = saprophytic Nisseria. See Table 1 for other abbreviations.

	No	ST	PP	SA	P	SH	STP	EC	PR	C	HI	EN	STA	SV	SN	SC
	Potentially pathogenic flora												Saprophytic flora			
1 Atelectasis	38	13	17	57	8	7	4	5	1	1	2	0	12	19	22	4
2. Infiltratio pulm. tbc.	26	7	27	27	3	9	8	0	1	0	2	0	0	9	12	6
3. Adenitis hill tbc	16	1	11	26	3	4	1	1	0	0	1	0	3	8	8	7
4 Pneumonia caseosa	4	0	3	1	1	1	1	0	0	0	1	0	0	1	0	0
5. Tbc. miliaris	3	1	3	1	1	1	0	0	0	0	1	0	0	1	1	0
6. Pneumonia and st. post	12	1	7	8	1	1	1	1	1	0	1	1	0	4	2	0
7 Bronchiectasia	10	0	14	12	1	4	2	2	0	0	5	0	0	4	4	4
8. Bronchitis	5	3	0	2	0	0	0	0	0	0	0	0	0	1	1	0
9 Others	6	0	8	7	1	3	3	0	0	0	1	0	0	3	1	0
Total	1 0	26	86	141	19	30	20	9	3	1	14	1	15	50	51	21
Total specific	87	22	57	112	16	22	14	6	2	1	7	0	15	38	42	18
Total non-specific	33	4	20	29	3	8	6	3	1	0	7	1	0	12	8	4

phytic flora In the group with a total of 33 cases of non specific diseases there were 12 cases with the diagnosis of bronchopneumonia, pneumonia, or st post pneumoniam, 10 cases of bronchiectasiae and 5 of bronchitis. The remaining 6 cases in this group included diagnoses such as abscessus pulmonum mediastinitis, tumor pulm., etc The most frequently isolated causative agent among the potentially pathogenic agents in this group was streptococcus haemolyticus, as it was in the group preceding it This agent was isolated in 8 cases it was followed by *Haemophilus influenzae* in 7 cases (5 of which were bronchiectasiae) staphylococcus pyogenes aureus in 6 cases, *E coli* in 3 cases, and enterococcus and proteus in one case each. Findings of saprophytic flora were far more frequently present than they were in the group of patients suffering from specific pulmonary diseases.

Sinuses

Total number of patients examined	110
Total number of samples examined	246
1 Punctate only	10
2 Lavage with saline only	ı
3 Lavage with bouillon	19
4 Nasal swab + lav bouillon + saline	08

Technique of collecting secretion from sinus Two puncture needles the liquid is injected through one and the contents of the sinus are collected through the other

A total of 246 samples from 110 patients suffering from acute or chronic

TABLE 3

See Tables 1 and 2 for abbre l tions.

	No.	Potentially pathogenic flora										Saprophytic flor		
		ST	PP	SA	P	SH	STP	EC	PR	C	HI	STA	SV	SC
Puncture only	16	9	5	4	0	0	2	0	0	0	3	1	1	2
Saline lavage only	7	3	2	2	0	1	0	0	0	0	1	0	0	2
Bouillon lavage only	19	3	13	5	1	4	4	1	0	2	1	4	1	0
Total	42	17	20	11	1	5	6	1	0	2	5	5	2	4
Nasal swab	68	35	16	18	2	2	4	1	1	2	4	9	4	5
Bouillon lavage	68	34	14	18	2	2	2	4	1	2	1	7	0	11
Saline lavage	68	52	4	13	1	0	0	2	1	0	0	3	0	10

sinusitis maxillaris were studied (see Table 3) Bacteri logical examinations were performed on punctates from the maxillary sinus—either samples obtained by puncture or material obtained by lavage with sterile saline (S.S) or sterile glucose bouillon. Sixteen samples of punctate were collected, 7 samples of lavage with sterile saline and 19 samples of lavage with sterile glucose bouill n. In 68 patients 3 samples were taken concurrently (1) nasal swabs, (2) lavage with sterile glucose bouillon, and (3) lavage with sterile saline By comparing these techniques of sample collection, we endea oured to disco er the best technique f recovering samples from the maxillary sinus.

Among the 16 cases in which th punctate only was taken, the samples collected from 9 patients remained sterile, and from the samples of 5 patients potentially pathogenic causative agents were isolated twice sta phylococcus pyogenes aureus and three times *H influen.ae* Saprophytic flora was found in 4 cases.

In the group of 16 patients from whom samples were recovered by means of sinus l age with saline there were 5 sterile samples, 2 of which contained potentially pathogenic flora, i.e. streptococcus haemolyticus and *H influenae*

In the group of 16 patient from whom samples were recovered by means of lavage with st rile bouillon th number of sterile samples was as low as 5 Potentially pathogenic flora was found in 13 and saprophytic flora in 5 Am ng th potentially pathogenic flora found there were streptococcus haemolyticu nd staphylococcus pyrogenes ureus 4 times each, proteus twice and pneumococcus, *H influenzae* and *E. coli* once each

A alread mentioned, in 68 patients 3 examinations, i.e nasal swabs, l ag with glucose bouill n, and lavage with saline were carried out imultaneously The sampl s taken from the nasal swab and by lavage with teril glucose bouillon equ lled each other in their number of sterile samples—3ɔ and 34, respectively the samples containing potentially patho-

genic flora also were almost equal in number i.e. their ratio was 16 14 Of the 68 samples taken by means of sterile saline lavage 52 were sterile potentially pathogenic flora was found in 4 samples only and saprophytic flora in 13 samples

Comparison of the results obtained in samples recovered by means of nasal swabs and sinus lavage with sterile bouillon showed that the same causative agents were not always isolated That is to say while *H influenzae* was isolated from nasal swabs in 4 cases and staphylococcus pyogenes aureus in 21 cases, the samples obtained by means of lavage with sterile bouillon fairly often contained the coliform group of *E coli* and proteus. Only once did we succeed in isolating *H influenzae*—hence we can only assume that contamination of the medium had probably occurred in the course of the intervention. The remaining bacterial flora mainly remained represented in equal numbers. We believe that these two methods should be employed in the bacteriological examination of the sinus if the puncture has been positive the punctate alone should be analyzed, if negative lavage with sterile bouillon should be carried out. The lavage with bouillon must always be followed by lavage with saline in order to remove from the sinus an ideal medium for bacteria.

CONCLUSION

On basis of our investigations, we may conclude that swabs from the ear in acute otitis media have a reduced diagnostic value if suppuration is not fairly abundant Bacterial flora sensitive to penicillin predominates. In chronic inflammation the flora is mixed and broad spectrum antibiotics produce the best effect In various pathological conditions of the tracheobronchial tree the flora varied greatly—hence only a combination of several antiobiotics can produce certain results. Only the punctate or lavage with bouillon should be used in bacteriological examinations of sinus infection, for if lavage with saline is used the picture of infection with *H influenzae* can easily be dissimulated. Concurrent bacteriological examinations of the nasal cavity and the maxillary sinuses have not yielded equal bacteriological findings—which speaks against the opinion that the bacteriological findings in the nasal mucosa can be a bacteriological indicator of the type of infection in the paranasal cavities.

ZUSAMMENFASSUNG

Insgesamt wurden 516 Fälle von Erkrankungen des Mittelohrs, der Nase Nebenhöhlen und spezifische und unspezifische Lungenerkrankungen bakteriologisch untersucht Auf Grund unserer Untersuchungen können wir feststellen dass der Abstrich aus dem Ohr bei akuter Mittelohrentzündung einen geringeren diagnostischen Wert hat, wenn die Sekretion nicht starker ausgeprägt ist. Es überwiegt die auf Penicillin reagierende bakterielle Flora Bei chronischer Entzündung ist diese Flora gemischt und die beste Wirkung erzielen Antibiotika mit weitem Spektrum Die bakterielle Flora ist bei verschiedenen pathologischen

Zuständen des tracheobronchialen Baumes sehr verschieden und nur eine Kombination mehrerer Antibiotika kann erfolgreich wirken Bei bakteriologischen Untersuchungen der \ be höhleninfektio mussen wir entweder da reine Punktat oder ein Waschung mit Bouillon gebrauchen, weil uns sonst bei Waschungen mit Kochsalzlösung oft das richtige Bild d r bakteriologischen In fektion mit Haemophilus influenzae verborgen bleibt. Di parallelen bakteriologischen Untersuchung n der \ase und der Ma\illarhöhlen ergaben nicht die gleichen bakteriologischen Befunde, was gegen die Meinung spricht, dass der bakteriologische Befund der \asenschleimhaut ein bakteriologischer I dikator der Paranasalinfektion sein kann.

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TEMPORARY ARTERIAL OBSTRUCTION

Effect on Perilymph Oxygen and Microphonics

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The principal source of oxygen to the cochlea is the arterial blood supply When blood flow (oxygen delivery) is stopped the reserves of oxygen in the cochlea are very limited and begin to fall in a few seconds. The oxygen reserves drop in a linear fashion and are exhausted in about 100 seconds. The oxygen reserve in the cochlea can maintain normal cochlear function (microphonic output) for only a few seconds after blood flow stops. After this cochlear function drops reaching a 50% level in 40 seconds. With return of blood flow cochlear oxygen tension recovers in a linear manner while cochlear function recovers more rapidly Full recovery of normal blood flow normal oxygen reserves and cochlear function can be obtained even after 8 minutes of obstruction of the internal auditory artery When blood flow resumes the initial velocity may be two to three times normal. In a short period (i.e. 100 seconds) depending on the duration of the obstruction, blood flow velocity returns to normal. This is an indication of the capacity of this vascular bed for autoregulation

Significant interrelations between blood flow oxygen availability and function have been found in the brain, heart kidney and skeletal muscle (Hirsch *et al.*, 1961 Chance *et al* 1962 Mayer *et al* 1962 Sayen *et al.*, 1958 Kramer & Deetjen, 1964 Stainsby & Otis, 1964 Lassen, 1964) Additional information about cell function has been obtained with locally perfused organs and in excised tissues in relation to oxygen utilization, substrates and enzymes involved, and function, i e brain slices and homogenates, nerves (Gerard, 1938) sympathetic ganglia (Larrabee & Bronk, 1952) heart muscle cell suspensions, etc Finally knowledge of *in situ* rapid intracellular changes in respiration (cytochromes in mitochondria) associated with anoxia apnoea and EEG changes have been obtained by use of microspectrophotometric methods (Chance *et al* 1962) The role of oxygen in cell function is thus becoming more clear

Some experimentally produced changes in cochlear blood flow and oxygen delivery have been related to changes in cochlear function and to changes

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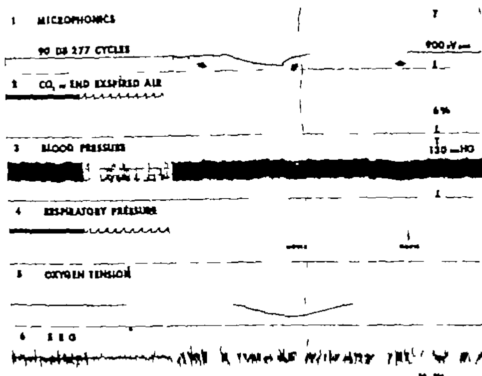


Fig. 1 Record f experiment n which cochlea blood flow was st pped for 90 sec.

in cochlear (perilymph) oxygen tension These resulted from obstruction to the inferior cochlear vein, anoxia, asphyxia, hypercarbia, hypertension etc. (Tsunoo & Perlman, 1965) The effect of sudden complete temporary interruption of all cochlear blood flow locally can also be examined because exposure and compression of the internal auditory artery is possible The method has been described earlier (Kimura & Perlman, 1958) In this way acute transient local ischa mia of the cochlea can be produced without altering the general physiological condition of the animal

For this study on the cochlea the effect of short periods of arterial occlusion (1–6 minutes) were studied since earlier experiments indicated that rapid severe reversibl changes in function result when cochlear blood flow is completely interrupted f r this time Local and systemic changes are recorded imultaneously (see earlier reports)

Findings

Blood flow

With prope orientation of the probe tip over the anterior inferi r cerebella artery immediate occlusion is obtained with a very small downward displac ment f the micromanipulator (even a vessel of 500 μ diameter

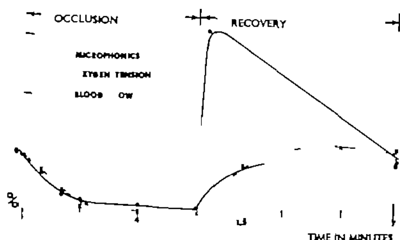


FIG 2. Chart indi ating simultaneous hanges in microphonic xyg n t nsi n and blood fl w rate during 6 minutes arteri l occlu ion nd after return f blood flow e pressed in per c nt of n rmal preocclu ion alues

with a blood pressure of 100 mm Hg can be occluded with 0.3 g force) This occlusion is indicated by the abrupt cessation of blood flow in the exposed strial vessels. Similarly with the micromanipulator a small elevation of the probe tip restores the blood flow at once Occasionally flow did not return due to damage of the vessel at the site of compression

Of interest is the prompt onset of supernormal flow on release of obstruc tion The degree and duration of this period of supernormal flow was related to the duration of obstruction i e return to normal flow in 7 minutes after 6 minutes of arterial obstruction Immediately after release, flow rates may be two to three times normal The absence of hemorrhage indicated gross integrity of the vessel walls with return of flow The stagnant red cells, plasma, white cells and platelets were washed rapidly out of the strial capillaries, radiating arterioles, arteriovenous arcades and venules Some dilation of the vessels was seen during the period of supernormal flow All vessels remained filled during the brief periods of circulatory arrest

Cochlear function

Microphonic response from differential electrodes in the 3rd turn of the cochlea to a continuous non overloading acoustic stimulus (277 cycles 90 dB) With cochlear blood flow stopped, a normal microphonic response could not be sustained for longer than about 5 seconds Then the response began to drop rapidly at first so that within 40 seconds it was reduced to 50% of the initial value The response decline then became slower but was almost complete in 120 sec Some further small drop could be seen how ever throughout a 6 minute obstruction On return of blood flow—50% of the initial RMS value for microphonic output was rapidly restored in about 80 seconds Both the initial rapid rise in microphonic and the slower later rise appeared to be uninfluenced by the length of obstruction up to 6 min

TABLE 1 *Recovery of microphonics oxygen tension and blood flow after arterial occlusion*

Microphonics is expressed in μv rms, and oxygen tension and blood flow in per cent of base line.

Time, condition	Base line	Recovery min				
		0	1.5	3.0	5.0	7.0
1.5 min occl.						
Microphonics	340	100–200	300	340	340	
Oxygen tension	100	35	83	95	107	
Blood flow	100	280	270	128	102	
2.0 min occl.						
Microphonics	340	80–180	320	320	330	
Oxygen tension	100	15	83	115	125	
Blood flow	100	260	212	200	121	
4.0 min occl.						
Microphonics	340	40–120	260	320	320	300
Oxygen tension	100	10	190	120	130	120
Blood flow	100	310	272	240	171	97

Illustrated in Fig 2.

utes. The recovery of microphonic response with return of blood flow was thus more rapid than its decline when the blood flow stopped. In the recovery period, unlike blood flow rates, no supernormal values were observed in the microphonic response. Indeed, occasionally full recovery of the response after obstruction did not occur during the period of observation (30 minutes)

Oxygen tension (in the perilymph of the scala vestibuli of the basal turn measured with a bare tipped 70 μ polarographic platinum electrode)

Oxygen tension began to drop almost immediately after blood flow stopped and continued to decline in a rather linear fashion for about 120 seconds when a plateau was reached corresponding to zero oxygen tension as subsequently determined with nitrogen breathing. The values were recorded a percent change, taking the level with air breathing and normal blood flow a 100%

A 50% decline in oxygen tensi n was reached in about 40 to 50 seconds. On return of blood flow the oxygen tension rose in a similar manner to its drop, reaching the 50% level in about 40 seconds. This recovery rate appeared to be independent of the duration of the obstruction up to 6 minutes. The 100% level w s reached in about 100 seconds fter return of blood flow A small degree of supernormal oxygen tension (13%) occasionally followed thi and continued f r 100 to 200 seconds depending on the dura tion of th bstruction.

DISCUSSION

Cochlear blood flow

The behavior of the strial vessels and their contents during arrest and return of cochlear blood flow has been described in earlier experiments (Perlman *et al* 1959)

The larger cochlear arteries probably dilate in response to occlusion of the feeding vessel (internal auditory artery) and this results in a brief period of supernormal flow in the strial vessels on release of the probe The reaction is a manifestation of autoregulation and has been recorded in many vascular beds, i e brain, kidney heart Other evidence for autoregulation of cochlear blood flow has been obtained (Perlman & Yamada 1967) The mechanisms for autoregulation of blood flow are complicated and continue to be investigated.

Cochlear function

The oxygen remaining in the cochlea after sudden arterial occlusion could sustain cochlear function (normal microphonic output) for only a few seconds The output dropped (rapidly at first and then more slowly) in a manner as has been reported earlier (Perlman *et al* 1959 Konishi *et al* 1961) The dependence of cell function on oxygen supply has been widely studied The recent investigations by Chance *et al* (1962) and his collaborators has thrown much light on the state of respiratory enzymes in the cell and oxygen utilization.

The recovery time of the microphonic response was more rapid than the survival time Different enzyme systems controlling the decomposition and regeneration of metabolic reserves may be responsible for this

Cochlear oxygen tension

The manner of calibration of the oxygen electrode in the cochlea has been previously described. No rhythmic fluctuations in perilymph oxygen tension or in blood flow was noted before during, or after arterial occlusion Davies & Bronk (1957) Clark *et al* (1958) and Silver (1965) report spontaneous fluctuations in oxygen tension with electrodes on the cortex These were taken to indicate rhythmic changes in cortical blood flow

With cessation of flow zero oxygen tension levels were reached in about 120 seconds as all the oxygen of the cochlea was used up by the metabolizing cells. Much of the oxygen in the cell is used by the cytochrome system in mitochondria for respiration

The rate of exhaustion of oxygen in the perilymph after occlusion of the internal auditory artery appears to be slower to reach zero (about 120 sec) than in the heart muscle on occlusion of a branch of the coronary artery (30 seconds) (Sayen *et al* 1958) Davies & Bronk (1958) report that local occlusion of an artery in the cortex resulted in a severe drop in oxygen to a sustained low value (5 mm Hg) in one second

After pO values in the cochlea reach a zero level and complete arrest of blood flow continues no improvement can be obtained with the animal breathing 100% oxygen. Similarly if the internal auditory artery is permanently damaged as indicated by failure of blood flow to return on release of the probe, the pO_2 values are not changed by respiring the animal with 100% O_2. The recovery of pO_2 values in the perilymph and short period of supernormality with return of blood flow resembles that seen in arterial occlusions of the brain and heart. The recovery of oxygen tension on return of blood flow was more rapid in the heart (5 to 10 sec) and brain (12 sec) than in the cochlea (perilymph) 50–90 seconds.

ZUSAMMENFASSUNG

Die hauptsächliche Quelle des Sauerstoffes der Cochlea ist der arterielle Blutstrom. Wenn der Blutstrom (die Sauerstoff Zugabe) el gehalten wird, ist die Reserve so gering, dass es in wenigen Sekunden abfällt. Die Sauerstoff Reserve fällt in lin enformiger Weise ab und ist in ungefähr 100 Sekunden verbraucht. Diese Sauerstoff Reserve in der Cochlea erhält die normalen microphonisch Potentiale der Cochlea nur für einig Sekunden nach der arteriellen Okklusion. Dann fällt die Funktion der Cochlea 60% i 40 Sekunden.

Nach dem Zurückkehren des Blutstromes stellt sich die Sauerstoff-Spannung der Cochlea wieder in einer linienförmigen Weise ein, wogegen die Funktion der Cochlea l l schn ller wiederhergestellt ist. Sogar nach 6 Minuten Okklusion der I ternal Auditory Arterie kann normaler Blutstrom, normale Sauerstoff Reserve und normale Funktion der Cochlea wieder fortsetzen. Wenn der Blutstrom wieder anfängt, kann die anfängliche Velozität zwei oder drei mal normal sein.

In einer kurzen Zeit (d. h., sek.) welches von der Länge der Okklusion abhängt, kehrt die normale Blutstrom-V lozität zurück. Das deutet auf die autoregulation Fähigkeit dieser Gefässe.

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EOSINOPHILIC PAPILLARY CYSTADENOMA OF THE LARYNX

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Only small number of reports on Eosinophilic Papillary Cystadenoma of the larynx has been made. The article describes two more cases. Treatment of the tumour is discussed.

Medical literature contains, as far as can be ascertained, reports on only 11 cases of eosinophilic papillary cystadenoma of the larynx. These cases have recently been presented by Steglich-Petersen (1968) who points out the difficulty in view of the small number of cases known, of making any definite conclusion regarding prognosis and treatment. In view of this it seems to be justified to present two more cases, one of which has been followed up for more than 3 years.

Case 1

A 66-year-old male who consulted us in 1963 on account of increasing hoarseness during 2 years. The examination revealed an oedematous, pea-sized, reddish polyp pendulating between the vocal cords. It was attached at the anterior commissure above the left vocal cord, and could be extirpated endoscopically. The histological diagnosis (elsewhere) was a benign polyp of the larynx, but it was also remarked that oncocytes were observed here and there. A follow up examination about 2 years later showed a local recurrence but, in addition, both the aryepiglottic folds were dotted with diminutive growths, one of which was removed for histological examination. Six months later a bean-sized tumour was extirpated at the anterior commissure under the right vocal cord. No progress has been observed at further check up during the last year however there is still a diffuse spread of minute growths over the aryepiglottic folds, the arytenoid region and the laryngeal surface of the epiglottis. On two further occasions, biopsy has been made from these.

In all seven biopsies during the period 1963–1966, microscopic examination revealed a typical papillary cystadenoma which, like the cases described in earlier published reports, was found to be made up of single-layer or two-layer eosinophilic (plerinophilic in a haematoxylin van Gieson-stained section) predominantly large-celled epithelium, with more or less pronounced granulation of the cytoplasma. The epithelium formed both cysts and



FIG. 1 Papillary formation just outside th main cystic tum or Case 1 Haemat xylin-van Gieson 160

densely clustered papillary formations which here and there occurred also outside the cysts. The epithelium showed no atypia or other indications of malignancy

Case 2

A 59 year-old male who consulted us in 1961 for hoarseness during 6 months. Examination of the larynx revealed a pea sized polyp located subglottally in the anterior commissure. A biopsy from this rather hard polyp gave only fragmentary material and at the microscopic examination (elsewhere) the growth was interpreted as a benign laryngeal polyp Three months later further polypic material was removed, but this was not examined histologically In 1963, a recurrence, the size of a grain of rice was found in the anterior commissure This was extirpated but the specimen was not examined histologically In February 1967 i e 6 years after the first symptoms, the patient consulted us again on account of increasing hoarseness and upon examination a pea sized, broad based polyp was found subglottically at the anterior commissure This was removed and was found to be a typical papillary oncocytic cystadenoma of the same type as in case 1

DISCUSSION

In case 2, it must be stated that only the extirpation in February 1967 can be regarded as radical and also that since there is no histological proof

that the same tumour was present at the different occasions of examination, it is still not possible to judge with any certainty whether or not there is a tendency for recurrences in this case. In case 1 on the other hand, the histological examination clearly revealed that the same type of tumour is present all the time The local spread which has occurred is of particular interest In the report from the first operation it is stated that "the stalk of the polyp is very hard and difficult to remove in its entirety" Among 31 cases of cystadenoma of the larynx described in medical literature there are seven in which the tumour contains oncocytes, large eosinophilic epithelial cells with granules in the cytoplasma. In no less than four of these cases has a local recurrence occurred, a circumstance which has not been found in those cystadenomas which lack oncocytes. In our first case, which belongs to the former morphological group, the tumour actually recurred, even in multiple form. The papillary glandular proliferation on the outside of the cyst in the case described above distinguishes the tumour from an ordinary retention cyst. We have gained the impression that tumours containing cells like those described abo e behave similarly i.e. have a marked tendency to recur especially in cases with pronounced extracystic, papillary glandular proliferation. In these patients, radical removal of the adenoma should be attempted in the first instance. If a local relapse occurs despite this, the therapy should be conservative, with check ups at frequent inter vals, as long as the respiratory passage is not interfered with. Radical surgery resulting in laryngeal invalidity cannot be considered justifiable. Malignancy has not occurred in any of the cases described hitherto. In some cases, there has instead been some tendency to stagnation of the tumour growth.

ZUSAMMENFASSUNG

Nur wenig Arbeiten über Eosinophile papilläre Cystadenomas im Kehlkopf sind geschrieben worden. Diese Arbeit beschreibt noch zwei Fälle. Die Behandlung wird diskutiert.

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MEATOTYMPANOPLASTY WITH SPECIAL REFERENCE TO THE POSTOPERATIVE HEARING

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We introduce our experience in "meatotympanoplasty", a tympanoplastic operation with the preservation of the ear canal. The outline of the procedure is elevation of the skin of the ear canal from the bony wall as a pedicule tube, retrograde removal of the bridge portion as needed, exenteration of the mastoid pathology by retroauricular approach, reestablishment of middle ear conductive system, covering the ossicles with the connective tissue flap and repositioning the skin of ear canal over the flap. Postoperative drying of the ear canal is attained early and the after care is unnecessary. It is also emphasized that the preservation of the normal ear canal is the method of choice from the standpoint of hearing gain. A favorable effect of the preservation of the normal ear canal upon the postoperative hearing was substantiated by clinical and experimental studies. Clinically, the hearing was compared before and after packing the posterior fossa of the tympanoplastically operated ear canal. Experimentally, the effect of the posterior fossa upon the resonance of ear canal was studied using a plaster ear model and dissected human temporal bones. These experiments confirmed that the normally constructed ear canal improved the hearing in higher frequencies by about 20 dB in comparison with that in the presence of posterior fossa.

It has been more than 15 years since tympanoplastic operations were first introduced by Wullstein (1952), Zöllner (1951), Goto (1953) and others. In a span of 15 years, a tremendous amount of fundamental and clinical work has contributed to the steady advancement of the art. We believe that the Japanese contribution in this field has been as valuable as those of European and American countries because we have a comparatively larger number of cases of chronic otitis media.

Considering the anatomical variability of the middle ear and the diversity of local findings, it is easily understood that no stereotyped technique of tympanoplasty can be applied to every individual case. Difficulty in tympanoplasty arises because one is always faced with two problems at the same time, namely exenteration of pathology and improvement of hearing, the aims of which are often incompatible.

The authors have reported in the past several years on tympanoplastic operations which are more or less original as to the techniques of hetten

plastik columella interposition myringoplasty and preservation of the bony ear canal

Among them what we call meatotympanoplasty a tympanoplasty with the preservation of the bony ear canal is our favorite technique and is the subject of this paper It has been proved to be an excellent technique in view of postoperative hearing gain and easy or almost unnecessary after care when *combined with the usage of connective tissue as an ear drum* substitute.

Meatotympanoplasty

We have practised this operation since the end of 1960 The primary concern of this operation is a morphological integration of the external ear canal This is achieved by elevating the skin of the ear canal as a pedicle tube exenterating the middle ear mastoid cell pathologies, reconstructing the sound conductive system and then repositioning the skin on the preserved bony ear canal Therefore, our meatotympanoplasty can be called a "meatus preserving operation"

A method intending to preserve the bony ear canal is by no means new Bárány applied it to the radical operation in 1923 Bárány left the skin of the ear canal in place while the posterior wall of the bony ear canal being taken down It can be said that there are two reasons which have stood in the way of further development of Bárány's method. The first is that before the advent of antibiotics, the method could not radically control the inflammation The second is that the large mastoid cavity made by the radical operation was less secretory than that made by the present tympanoplastic operation The reason for the second must be that the mastoid cavity of radically operated ear is lined with the extended skin of the ear canal, while the cavity made by tympanoplastic operation is covered *with a heterotopical* skin flap from the retroauricular region anterior thigh or upper arm, etc., and that the heterotopical skin is naturally different from the skin proper in glandular secretion, desquamation, resistance to bacteria and other stimulations.

It is quite reasonable that the preservation of a "fossaless" ear canal in tympanoplasty has received world wide acceptance, when we consider how troublesome it is to keep a large mastoid cavity clean and dry against retarded epithelialization, accumulation of desquamated mass, and repeated secretory dermatitidis. Wullstein and House also insist upon the preservation of the ear canal A number of different measures against the posterior fossa are not mentioned here One of such measures is the preservation of the posterior wall of the external ear canal (Naito, 1961 Ouchi, 1960 Aimi 1963 Muta, 1964 Austin 1961 McLaurin 1961 Brunner 1959 Myer 1960 Jansen 1963 Schnee, 1963 Farrior 1962 Ruyra 1962 Palva 1963 and others)

We would like to outline the operative procedures of meatotympanoplasty (Fig 1)

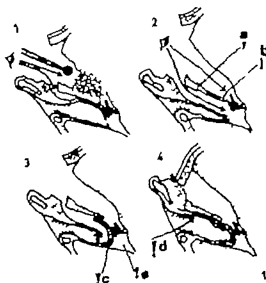


Fig. 1 Meatotympanoplasty of our rece t practice (horizo tal section)

1 Retroauricular incision along the root of the pinna.

2 Elevation of the skin of the ear canal down to the ear drum as a pedicle tube.

3 Total exenteration of pathological mastoid cells with the posterior wall of the ear canal preserved (a!)

4 Management of the ossicular chain and oval window after the retrograde removal of the bridge portion, when necessary (b!)

5 Biting off the postero-inferior portion of the ear canal for better visualization and treatment of the hypotympanum and round window

6. Management of the tubal orifice and its surroundings.

7 Filling up the middle ear cavity with pieces of gelatin sponge moistened with antibiotic solution (e!)

8. Making new ear drum of a small, 1 mm thick piece of connective tissue from the fascia temporalis or its neighbourhood (c!)

9 Repositioning" of the skin sheath, its deep portion extending over the connective tissue flap (d!)

10. Packing the external ear canal with pieces of gelatin sponge.

11 Primary closure of retroauricular wound.

When h mostasis, and exenteration of the pathology are complete, tam ponad f the closed mast id cavity is not necessary Tubal air passage is begun from the 4th to 5th postoperati e day with minimal pressure. About 2 weeks post peratively the packing in the ear canal is removed, and thereafter it is kept without packing. Epithelialization down to the newly implanted ear drum becomes completed and the ear canal dry up in 2 to 3 weeks.

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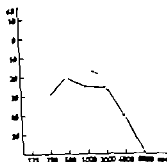


Fig. 3.

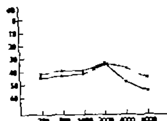


Fig. 4.

Fig. 3. Gradual improvement of postoperati hearing in fascia method. X——X preoperati hearing; X X, 3 weeks fter operation X X 6 months fter operation.

Fig. 4. A erage of pre- and postoperati hearing f 30 cases of tympanoplasty X —X preoperati ; X——X postoperati e.

tympanoplasty can be also performed with free skin flap At any rate so many people, so many tastes" is also true as to the techniques of tympanoplasty

Postoperative Hearing Gain

1 *Statistical observations* It seems quite controversial to evaluate each technique in terms of postoperative hearing gain, by putting many different cases together for the purpose of a statistical study since the preoperative threshold, status of the ossicular chain, pathology in the middle ear and mastoid cells and many other factors are different from one case to another

Supposedly the effect of the posterior fossa of the ear canal upon post operati e hearing is f minor importance in comparison with re-establishment of the middle ear conductive system Therefore, the effect of the

Table 1 *General comparison of sliding method" and fascia method" of meatotympanoplasty*

	Sliding method	Fascia method
Technique	Difficult	Easy
Skin graft	Occasionally needed	Unnecessary
Epithelialization	Slow	Fast
Postoperati perforation	Occasional	Seldom
Narrowing of the entrance of the ear canal	Occasional	—
Postoperati hearing gain	Early	Retarded
Postoperati care	Unnecessary	Unnecessary

This method is applicable to any of Wullstein s I to IV types on the condition that a certain special precaution should be exerted in placing the connective tissue flap for some cases of types I and IV The newly implanted ear drum substitute gradually becomes thinner to be of normal thinness in 3 to 6 months.

Comparison of Fascia method with Sliding method"

In the early practice of meatotympanoplasty we used an anteriorly slided pedicle skin flap to cover the middle ear cavity The skin is carefully elevated from the posterior bony wall after incisions are made at the upper and lower corner of the posterior canal wall almost down to the annulus tympanicus (sliding fore method) Thus shifted skin serves favorably as an ear drum soon after the operation since the skin is acoustically thin enough from the beginning On the other hand, the sliding fore method has following disadvantages

(*a*) It takes time and is not necessarily easy to elevate the skin of the ear canal down to and over the annulus tympanicus without perforating it.

(*b*) The thin ear drum substitute tends to occasional perforation due to postoperative fluid retention in the middle ear

(*c*) Skin graft is necessary to the area of skin defect in the posterior wall, where otherwise, natural epithelialization takes a fairly long time.

Fascia method brings about complete solution of those problems. However the ear drum substitute as thick as 1 mm retards postoperative hearing gain particularly in higher frequencies Hearing gain gradually manifests itself in 3 to 6 months

Fig 2 is the comparison of fascia method and sliding method in terms of hearing gain of 1 month after the meatotympanoplasty In such early postoperative days, sliding method is superior to fascia method.

Fig 3 is a demonstration of gradual improvement of hearing even 6 months after fascia method Such is often the case and indicates the necessity of further follow up

Table 1 is a general comparison of the two methods One might as well choose either of them according to the individual case encountered Our recent choice is fascia method in the majority of cases Naturally meato-

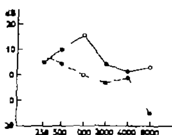


FIG. 2. Comparison of hearing gain by fascia method and sliding method (average of 10 cases each) O——O sliding method, O- - - -O fascia method

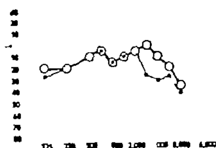


Fig. 7

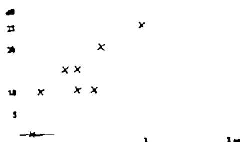


Fig. 8.

Fig. 7 Cha g of hearing before and after packing of posterior fossa with chicle. •——• Before packing; O——O after packing.

Fig. 8. Relation between the olume of posterior fossa and hearing gain t 3000 cps whe the fossa is filled p. 0 dB Pretreatment threshold.

3 *Experiments using plaster ear models and dissected human temporal bones* To make a quantitative analysis of the above-mentioned experiments, we performed the following observations using plaster ear models and dissected human temporal bones. Models of the pinna and ear canal were made of plaster A receiver was placed against the auricle and the sound pressure was measured with the condenser microphone which was set at the point corresponding to ear drum. The same observation was made with the models, the posterior wall of which was caved in a manner of classical tympanoplasty The volume of the fossa was one to four ml. The resulting hearing loss in higher frequencies was, as shown in Fig 10 in proportion to the volume of the posterior fossa.

The same experiment done in a free field gave essentially the same

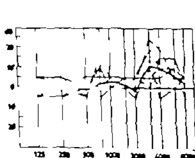
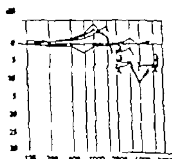


Fig. 9



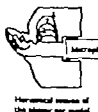


Fig. 10.

Fig. 9 Change i hearing after the packi g of the posterior fossa i 10 cases of tympanoplasty (thick line erage f 10 cases) 0 dB Pretreatment threshold.

Fig. 10. Relatio bet een the lume of the posterior fossa nd hearing loss in ear model experiment. The umbers 1 to 4 on the lines correspond t the ol me of the posterior fossa of 1 t 4 ml. 0 dB Sound pressure with the normal ear canal.

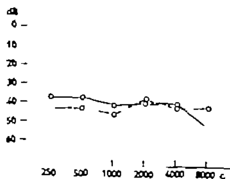


Fig 5

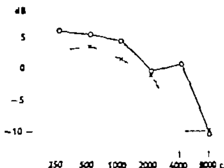


Fig 6.

Fig. 5 Av rage of pre and postoperative hearing of 20 cases of meatotympan plast O----O Preoperative O——O postoperative

Fig. 6. Comparison of h aring gain in two groups (20 cases each) 0 dB, No hange + gain of hearing – loss of hearing ×----× tympanopl sty O——O meatotympanoplasty

posterior fossa upon hearing cannot be duly evaluated when one merely compares the hearing of two groups, the group of meatotympanoplasty with normal ear canal and the group of classical tympanoplasty with a large posterior fossa

Admitting the above mentioned disadvantages, postoperative hearing of the two groups was statistically observed Materials were 20 cases of tympanoplasty and the same number of meatotympanoplasty which had been all operated upon in our clinic in 1963 (Figs. 4 5 and 6)

It was noted from the comparison that meatotympanoplasty is better as far as the improvement of hearing at 4000 cps is concerned. This result encouraged us to do the following experiments

2 *Clinical experiments* Mixture of various factors in the above-mentioned statistical observation makes it difficult to pick up the effect of the posterior fossa upon hearing in its pure form

As the next step therefore, we performed the following experiments The large posterior fossa of the ear canal, which had been operated upon according to classical tympanoplasty was filled up with chicle (the material of chewing gum) to such an extent that the ear canal was almost rebuilt but that the vibration of the ear drum was not disturbed at all and then the hearing was compared before and after the treatment The experiment, which in my opinion excluded many factors from the middle and inner ear might give an answer to the question.

Fig 7 is an example It shows a marked improvement of hearing in higher frequencies, 25 dB at 3000 cps and 20 dB at 4000 cps, while there was no shifting of threshold in lower frequencies.

The packing of the posterior fossa, however does not always favorably influence the hearing, but the general rule is that the larger the posterior fossa, the greater the hearing gain by its packing (Fig 8)

Fig 9 indicates the average hearing gain of 10 cases 15 dB at 3000 cps and 10 dB at 4000 cps

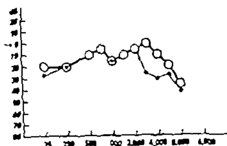


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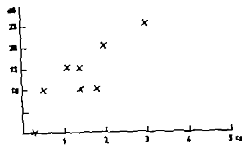


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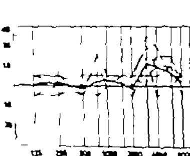


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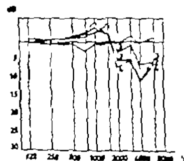
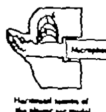


Fig. 10.



Horizontal section of the plaster ear model

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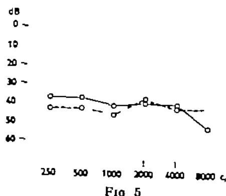


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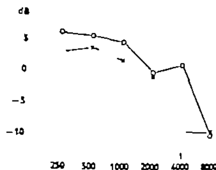


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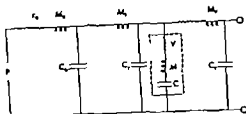


FIG. 15. Acoustic circuit of external ear canal. P Effective sound pressure; M C acoustic resistance, inertance, and capacity at the entrance of external ear canal M acoustic resistance, inertance, and capacity of ear canal; M C, acoustic resistance, inertance, and capacity of posterior fossa.

From an acoustic point of view the external ear canal is thought to be a tube and its acoustic circuit can be assumed to be as shown in Fig. 15.

The acoustic impedance caused by the air in the posterior fossa connects as a parallel circuit with the acoustic circuit of the external ear canal. (The acoustic impedance caused by the posterior fossa is encircled by a dotted line.)

It is reasonable to assume that this circuit system has a resonant frequency ($f=1/2\pi\sqrt{MC}$) and the sound near the resonant frequency is partly shunted, resulting in hearing loss. The volume of the posterior fossa made by classical tympanoplasty is maximum 3 ml, and the resonant frequency of the circuit happens to be in the range of 3000 to 4000 cps. That is how the conduction of the sound in that range is disturbed.

CONCLUSION

It is needless to say that a careful treatment of the middle ear and mastoid is important, but the maintenance of a normal ear canal is equally important from the viewpoint of postoperative care and hearing. As far as the management of the mastoid cells is concerned, some people insist upon radical exenteration of pathology the others prefer to treat as conservatively as possible and still others take an intermediate stand. In classical tympanoplasty desirable radical exenteration of mastoid pathology was necessarily accompanied by a large posterior fossa, which made postoperative care very troublesome.

More conservative management of the mastoid cells leaves the control of their inflammation largely to a natural healing process with minimal sacrifice of the ear canal. However this conservativeness inevitably involves some risk of recurrence of inflammation. The same holds true as to the management of middle ear pathology removal of pathological mucosa is occasionally followed by adhesion of the ear drum substitute to the promontory while preservation of the mucosa is not without the risk of recurrence of inflammation.

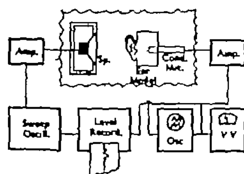


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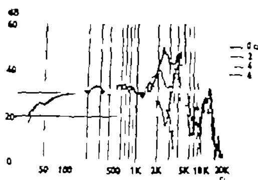


Fig. 12.

Fig. 11 Equipment for the test in a free field.

Fig. 12 Change of the sound pressure level at the ear drum in correspondence to the volume change of the posterior fossa.

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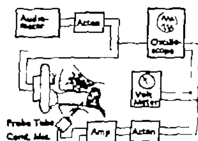


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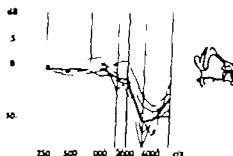


Fig. 14.

Fig. 13. Method of experiment using a dissected human temporal bo[ne]

Fig. 14 Effect of the posterior fossa upon hearing in dissected human temporal bones (thick line: average of fi[ve] cases) 0 dB Sound pressure with normal ear canal

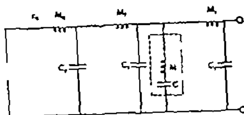


Fig. 15. Acoustic circuit of external ear canal. *P* Effective sound pressure; M_0 r_0 acoustic resistance, inertance, and capacity at the entrance of external ear canal; M acoustic resistance, inertance, and capacity of ear canal; M c, acoustic resistance, inertance, and capacity of posterior fossa.

From an acoustic point of view the external ear canal is thought to be a tube, and its acoustic circuit can be assumed to be as shown in Fig. 15.

The acoustic impedance caused by the air in the posterior fossa connects as a parallel circuit with the acoustic circuit of the external ear canal. (The acoustic impedance caused by the posterior fossa is encircled by a dotted line.)

It is reasonable to assume that this circuit system has a resonant frequency ($f=1/2\pi\sqrt{MC}$) and the sound near the resonant frequency is partly *shunted* resulting in hearing loss. The volume of the posterior fossa made by classical tympanoplasty is maximum 5 ml and the resonant frequency of the circuit happens to be in the range of 3000 to 4000 cps. That is how the conduction of the sound in that range is disturbed.

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It is needless to say that a careful treatment of the middle ear and mastoid is important, but the maintenance of a normal ear canal is equally important from the viewpoint of postoperative care and hearing. As far as the management of the mastoid cells is concerned, some people insist upon radical exenteration of pathology, the others prefer to treat as conservatively as possible and still others take an intermediate stand. In classical tympanoplasty desirable radical exenteration of mastoid pathology was necessarily accompanied by a large posterior fossa, which made postoperative care very troublesome.

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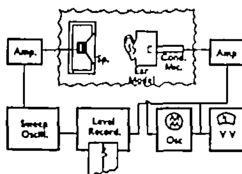


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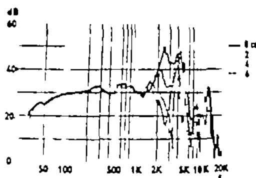


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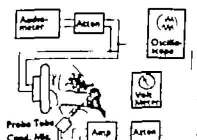


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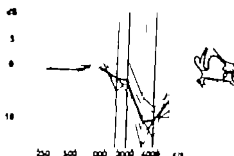


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Fig. 13. M thod of pe im t u i g di sected huma t n poral bon

Fig 14 Effect f the posteri f sa po i ri g t di sect d human t mpo l bo es (thick lin : average of fi e case 0 dB Sound pre ure with normal e n l

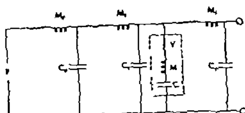


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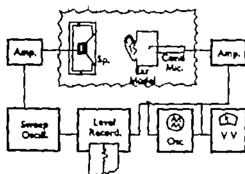


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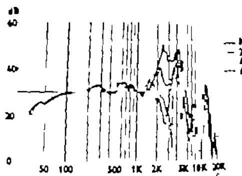


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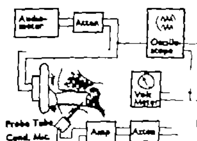


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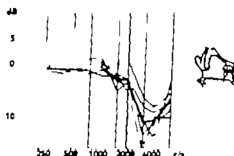


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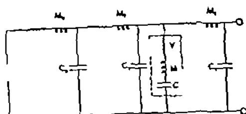


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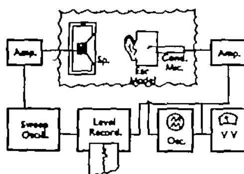


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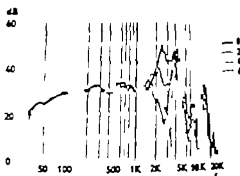


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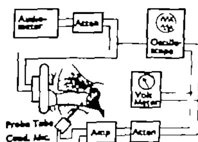


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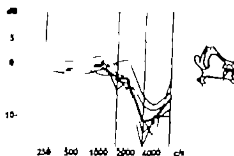


Fig 14

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dung des Trommelfells mit Bindgewebe-Transplantation und Reponierung der äusseren Gehörgangshaut auf bindgewebige Transplantate. Nach der Operation ist die Operationswunde schnell trocken und die Nachbehandlung ist unnötig. Es muss noch betont werden, dass die Erhaltung des normalen äusseren Gehörganges nach der tympanoplastischen Operation der wichtigste Punkt in Bezug auf Hörverbesserung ist. Der gute Effekt durch die Erhaltung des normalen äusseren Gehörganges über das postoperative Gehör wurde mit klinischen und experimentellen Versuchen bemerkt. Klinisch wurde das Gehör vor und nach Plombierung des "chiels" in der Operationshöhle der mastoidalen Zellen, d. h. ohne und mit dem relativ normalen äusseren Gehörga g geprüft. Experimental wurde der Effekt der Operationshöhle über Resonanzphänomen mit dem modellierten Ohr und menschlichen temporalen Knochen untersucht. Dieses Experiment hat gezeigt, dass normal gebildeter äusserer Gehörgang besseren Gehörgewinn, etwa 20 db, in hohen Frequenzen erzielt als der beim Zurückbleiben des Operationshöhle erfolgt.

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We feel that meatotympanoplasty gives a solution to the above-mentioned contradictory problems the pathological cells can be radically exenterated with no deformity of the ear canal and adhesive process of the middle ear cavity is avoided by packing the middle ear cavity thickly with pieces of gelatin sponge.

Meatotympanoplasty has of course its own problems to be solved. The first of them is the problem of a large dead space in the mastoid process. In general principle of surgery formation of a dead space is considered undesirable from the viewpoint of fluid retention and infection. But practically we have experienced no disadvantages such as rupture of retroauricular wound or secondary perforation of ear drum substitute Presumably this might be due to drainage through the eustachian tube and osseous structure of dead space wall

Further follow up is of course necessary to observe the destiny of a large mastoid cavity and its effect upon air conduction.

The second problem is the use of the fascia as an ear drum substitute The fascia used should be fairly thick so as not to be perforated easily The thickness retards the improvement of hearing until 2 to 3 months after the operation, as we have stated in the preceding pages. The fascia is also more susceptible to infection than skin flap particularly to infection from outside. We have experienced a few cases of infection of the fascia, which, however was all arrested in the early stage of superficial necrosis with the help of antibiotics and did not lead to perforation. This is why we leave pieces of geletin sponge moistened with antibiotic solution in the ear canal for 2 weeks or so

Admitting some disadvantages of meatotympanoplasty we are still considering it worth while to present our experiences in meatotympanoplasty because the method is superior to classical tympanoplasty in regards to the improvement of hearing as well as easy postoperative care. Even if the hearing gain attained by the method might not be of significant magnitude, our efforts should always be directed to the benefit of patients.

The history of tympanoplasty is short and there is a long way to go before 100% success can be attained We Japanese otologists have many more cases for tympanoplasty than Europeans and Americans, and we feel keenly the necessity for further research in this field

ZUSAMMENFASSUNG

Der Verfasser hat einige Erfahrungen von „Meatotympanoplastv d h der tympanoplastischen Operation unter die Erhaltung des äusseren Gehörganges berichtet. Die Operations-Techniken bestehen aus Ablösung der äusseren Gehörgangshaut als gestielltes Röhrchen aus dem äusseren Gehörgangsknochen notfalls retrograde Entfernung der äusseren Gehörgangsbrücke, Beseitigung des krankhaften Teils in den mastoidalen Zellen durch den retroauricularen Eingriff Wiederherstellung des Schalleitungs-Systemes im mittleren Ohr Neubil-

Acta oto-laryngologica 67 583–588 (1969)

EAR MALFORMATIONS IN CLEIDOCRANIAL DYSOSTOSIS

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A case is reported of a 12-year-old girl with familial cleidocranial dysostosis, pronounced ear malformations, and a conductive hearing loss. The right auricle was small and deformed and both external ear canals were atretic. Radiograms of the temporal bones showed dense calcification, atretic middle ears, and malformations of the auditory ossicles.

More than 600 cases of cleidocranial dysostosis have been described since the publication by Martin in 1765. Scheuthauer (1871) and Marie & Sainton (1897) have had their names associated with the syndrome, but today the more descriptive name cleidocranial dysostosis has been widely accepted.

The etiology of the disease is unknown but seems to be connected with a defect in the parental germ plasm as first suggested by Hultkrantz (1908). The majority of cases appear on a hereditary background but many examples of spontaneous, isolated occurrence have been given. The disease may be transmitted in a few generations and then disappear in the following generations (Witkop-Oostenrijk, 1957). The variety and inconstancy of the skeletal anomalies are prominent features of the syndrome.

The combination of hypoplasia of the clavicles and brachycephalia led to the assumption that bones of membranous origin were primarily affected (Fitchet, 1929). Later investigations have demonstrated that multiple, developmental malformations of the whole skeleton are usually present. Besides hypoplasia of the clavicles and delayed closure of the cranial sutures and fontanelles, reports have been given of prognathism, irregularities in dentition, structural abnormalities in vertebrae, sacrum, pelvis, femora, scapula, metacarpals, metatarsals and phalanges (Kalliala & Taskinen, 1962). A recent survey was given by Gorlin & Pindborg (1964).

Although a few cases of mental disease have been reported (Kilgore & Lasker 1946), no constant relationship to the dysostosis has been established.

In the present context the cranial deformities are of special interest. The skull is large and brachycephalic with pronounced frontal and parietal bossing. Due to delayed closure of the sutures, the fontanelles usually remain open and numerous Wormian bodies are formed. The bones of the face are underdeveloped except for the mandible which is long and prognathous. This disproportion of the skull and face in connection with the short body and drooping shoulders makes the general appearance almost pathognomonic. The palate is narrow and highly arched and may have a

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FIG. 2. The patie t's mother ( ) her twin sister (*b*) nd her younger sister ( )

she had been receiving orthodontic treatment for some time. Measuring 142 cm and weighing 37.5 kg, she was considerably smaller than the normal average for her age (158 cm/47 kg)

The familial occurrence was evident as the patient showed a striking similarity to her mother her twin sister and a younger sister (Fig. 2 *a b c*) On the other hand, her grandparents were normal and to the mother's knowledge there were no other cases of cleidocranial dysostosis in the f mily

A complete radiological examination showed all the typical cranial deformities mentioned above. The clavicles were hypoplastic having two small ossification centers each. A spina bifida was found from vertebra C 1–T 5 nd vertebra L 5 was also bifid. The pubic bone was incompletely ossified, but the long tubular bones as well as hands and feet were normal

There was no hist ry or symptoms of intellectual or emotional disturb-



FIG. 3. F ontal ( ) lateral (*b*) and posterior ( ) ew of the patient's right auricle.



Fig. 1 Th patient s gen ral appearance w typical of cleidocranial dysostosis.

submucous or complete palatal cleft Delayed and irregular dentition and supernumerary teeth are usually found The base of the skull is small often with incompletely ossified symphyses The short longitudinal diameter causes extensive arching of brow and occiput The small width of the cranial base makes the squamous portions of the temporal bones sit in a diagonal position, so that the mastoid processes are displaced in a medial direction and lie in a deeper location than normal The foramen magnum is large and turned forward, its level passing through the glabella The clivus is short with an abrupt decline (Hultkrantz, 1908)

In view of these pronounced cranial deformities it would hardly be surprising to find malformations of the petrous portions of the temporal bones with or without functional defects Nevertheless, no such examples can be found in the extensive literature on this subject and a thorough auditory and vestibular examination does not seem to have been carried out in any of the reported cases The following example of pronounced ear malformations in a patient with cleidocranial dysostosis is therefore presented

Case Report

A 13-year-old girl was admitted to our department with bilateral atresia of the external acoustic meatus and impaired hearing Her general appearance was typical of cleidocranial dysostosis (Fig 1) but also typically her physical condition had not called for any previous medical attention while

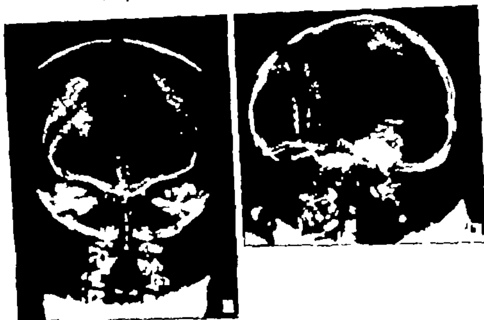


Fig. 5. Ordinary frontal ( ) and l teral (b) radiogram of the crani m.

be identified The inner ear was normal and the internal acoustic meatus of a normal caliber

On the left side the external meatus was only slightly diminished but the middle ear was almost as small as on the right side. The ossicles on the left side were lso smaller than normal but the handle of the malleus and the long process of the incus could be identified as small prominences. The inner ear and the internal meatus were normal

An ordinary radiogram of the cranium (Fig. 5 a b) as well as the tomograms mentioned above sh wed two characteristic findings. On both sides the mast id cells were bsent so that the petrous portions appeared densely calcified Fig. 5 a also hows the typical slope of the two temporal bones.

Pure tone audiometry howed a bilateral conductive hearing loss (Fig. 6) The peech reception threshold was 4 dB on the right side and 25 on the left By means of acou tic impedance examination the middle ear pressure wa found to be normal on the left side and a middle ear reflex was present. On the right side the ear drum impedance remained unchanged with varia tion of the pres ure in the ear canal as evidence of accumulati n of fluid in the middle ear

Cal ric estibular tests were n rmal.

From the dental examination it was concluded that the maxilla was underdeveloped with a highly arched, narrow palat while the mandible was normal for the age The dentition was delayed but there were no supernumerary teeth.

The patient two sisters and their mother showed the radiological find-

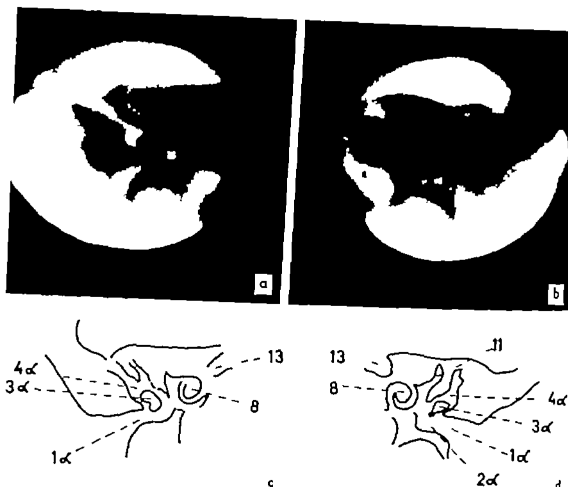


FIG. 4 Frontal tomogram of the right (*a*) and l ft (*b*) ear Nomenclature f the dia grams (ight, *d* l ft) 1 External acou tic meatus 2, tympanic ca ity 3, auditory ossicl s 4 mastoid antrum 8, cochlea 11 superi semicircular can l 13, int rnal a oustic meatus. Alpha, pathol gical chang (e.g deformed ssicl s, atresia tc.)

ances and the patient seemed socially well adjusted despite her cosmetic handicap Sexually she was normally developed and had had regular menstruation for almost one year

The right auricle was small, coneshaped and turned forward at an angle of 90 with the side of the head (Fig 3 *a b c*) The right external meatus was too narrow for a satisfactory otoscopy but with an operating microscope the tympanic membrane could be seen It was much smaller than normal and no landmarks could be identified.

The left auricle was normal and the diameter of the left external meatus only slightly under average The tympanic membrane showed an unusual protrusion of the handle and lateral process of the malleus which was also located close to the posterior metal wall

On a frontal tomography (Fig 4 *a b c d*) the right external meatus appeared narrow and the middle ear smaller than normal The ossicles seemed to form a small irregular bone condensation in the attic and neither the handle of the malleus nor the long process of the incus could

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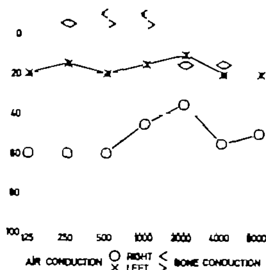


FIG. 6 Pure tone audiogram on dmi ion

ings typical of cleidocranial dysostosis but they had no middle ear mal formations and otoscopy and hearing were normal

The serous otitis on the patient s right ear responded promptly to insufflation and administration of ephedrine nasal drops with a subsequent hearing improvement of almost 20 dB

In view of the temporal bone malformations described above the remaining hearing loss seemed only moderate Consequently a surgical attempt on further hearing improvement did not seem advisable

A plastic operation on the right auricle was planned but for practical reasons it was postponed.

ACKNOWLEDGMENT

The author wishes to express his gratitude to Dr H Rovsing, the Radiological Department, Rigshospitalet for providing the radiograms and diagrams.

ZUSAMMENFASSUNG

Bei einem 13-jährigen Mädchen mit Dysostosis Cleidocranialis wurde ein kon duktiver Hörverlust und schwere Ohrenmissbildungen gefunden Die rechte Auri kula war klein und missgebildet und Meatus akustikus externus war auf beiden Seiten atretisch Röntgenaufnahmen der Felsenbeine zeigten dichte Sklerose atretische Mittelohre und Missbildung der Gehörknöchelchen

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among others have performed fundamental anatomical investigations, many question still remain which cannot be answered before we know more bout the anatomy of the system

What is it like compared to other known species, man or cat, and even more interesting, compared to the whales, which also employ echolocation

The present investigation tries to add to our knowledge by applying two methods recently developed for investigation of the cochlea and the cochlear nuclei.

MATERIAL AND METHODS

The material consists of 20 specimens of the microchiropterae Plecotus auritus collected from an old church tower in Norway. They were anesthetized by ether until soundly asleep and then decapitated. Ten animals were used for preparation of the cochlea, the others for preparation of the brain and the VIIIth nerve. For the preparation of the cochlea the osmium tetroxide fixation and the surface preparation technique described by Engström *et al.* (1966) was applied. The brains were fixed in 10% Formaline, sectioned in 10 microns and stained with Thionine. The method described by the author (Hall, 1964 and 1967) was then applied for counting the number of cells and for computing the volume of the cochlear nuclei.

The part of the medulla containing the cochlear nuclei was sectioned in 10 micron sections, every 10th section was magnified and the outlines of the nuclei drawn on paper employing a Bausch & Lomb projection apparatus. Then their areas were determined using a planimeter and the volume calculated by simple mathematics.

OBSERVATIONS

Although this paper primarily deals with the cochlea and the cochlear nuclei, some data collected concerning the external parts of their sound perceiving system might also interest the reader. As indicated in their names, the pinnae of the Plecotus auritus were enormously large, they measured up to 3 cm in length, whereas the body including the head, was only 7 cm long (Fig. 1). The width of the skull was 9 mm, and the bullae occupied 3 mm on either side (Fig. 2).

The oval ear-drum stretched out within the bulla measured 2×3 mm, thus occupying one-third of the transverse diameter of the head (the ear drum in man measures 9×8 mm).

The ossicles of the middle ear are situated as in other mammals, but some details may be significant for the perception of higher frequencies. The head of the malleus is comparatively great, its shaft broad, more or less formed like a plate (Fig. 3).

The long leg of the incus is gracile, there is a distinct processus lenticularis, articulating with a small, thin and fragile stapes whose crurae, as in homo, are inward concav.

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THE COCHLEA AND THE COCHLEAR NUCLEI IN THE BAT

J G HALL

From the Anatomical Institute and the E N T Department University of Oslo, Norway

The outer and inner ears and the cochlear nuclei in the bat Plecotus auritus are described. The author has collected 20 specimens of this animal, whereof 10 were used for preparation of the cochlea, applying the method of H Engström In the other half the cochlear nuclei were prepared following the method described by the author in 1964 The volume of the cochlear nuclei and their number of nerve cells are computed and the distribution of the primary nerve fibres is described. The primary auditory centers in the bat are compared with those of the cat the whales and homo

Some of the nocturnal carnivorous microchiroptera are wholly dependent upon their hearing as echolocation provides their means for the location of obstacles and prey (Spallanzani 1794 Hartridge, 1920 Galambos and Griffin 1940 Dijkgraaf 1946 Møhres, 1952 Griffin 1958 Grinell 1958) The echoes result from cries of high intensity produced in a highly specialized larynx (Pye 1961)

The intensity of their calls has been measured 10 cm in front of their mouth and may at that point reach 60 Dyn or approximately 110 dB They usually employ a frequency range from 20–120 kilocycles/sec, thus practically inaudible to man The frequency of the call also in some species vary from high to low declining about one octave during each call When not flying, they emit only a few cries, about 8/min but during flight or prey catching they may emit up to 200 cries/sec As they are able to detect their own echoes even in caves where several hundred bats are shrieking at the same time and even in white noise covering their own spectrae, a highly specialized discriminative system must be involved Pye (1960) has proposed that they must be listening to the beat note occurring between the call and the echo

Griffin (1958) has also shown in Vespertilionideae that they employ different frequency modulations in each cry and that the number of cries sec may vary considerably increasing when nearing an object The highly specialized forms of the outer ears, especially in Vespertilionideae and the quick movements of the outer ears (Rhinolophidae) certainly also represent suitable means for detecting the echo The investigations cited above have thrown some light upon the function of the extraordinary auditory system in bats However although Poljak (1926) and Reysenbach de Haan (1958)

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Fig. 3. The middle ear ossicles. Note the flat, plow-like shape of the hammer. 15.

brane 6.3 mm, the latest number found by measuring on a photograph after removing the basilar membrane in sections (Fig. 4). The length of the basilar membrane in man is 30–35 mm (Bredberg, 1968).

The organ of Corti is built as in other mammals, with three distinct rows of outer hair cells and one row of the inner hair cells. The outer hair cells were long and slender and the inner hair cells short, plump, flasklike, with a great round nucleus. A counting under the microscope showed that 50 of the inner hair cells occupied the same space as 60 in one of the outer rows (Fig. 5).

The hairs, when seen from the surface, show the same "W" form as in man and cat (Fig. 6).



Fig. 4. Preparation of the cochlear membrane. 20.

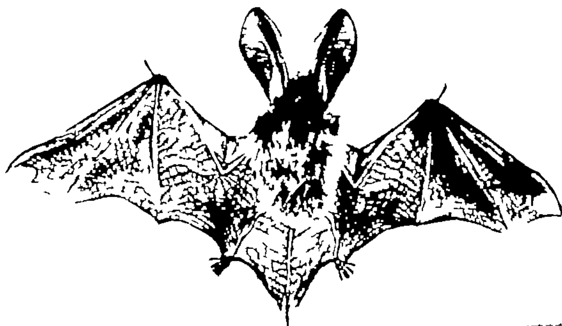


Fig. 1 Plecotu auritus, th most common bat in Norw y Note the long ears.

The cochlea has 2 $^{1}/_{4}$ coils, whereof the basal one is by far the greatest Its diameter measured with a micrometer in the microscope was 2.33 mm (in man 7 mm) whereas the upper coil measured only 1 27 mm

The height of the cochlea was 2 7 mm and the length of its basilar mem-



Fig. 2 Upper jaw nd cochlea On th right ide, th b ll nd th -drum l isible. On the left side the bulla i rem ed, and th cochlea il insid 10.



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Fig. 4 Preparation of the cochlear membrane. 30

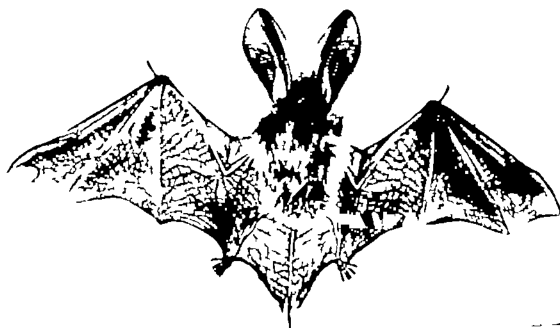


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FIG. 2. Uppe j w and cochlea On th right side, th bull nd the ea -drum i isible. On the l ft side the bulla s rem ed, nd the cochl is in id 10.

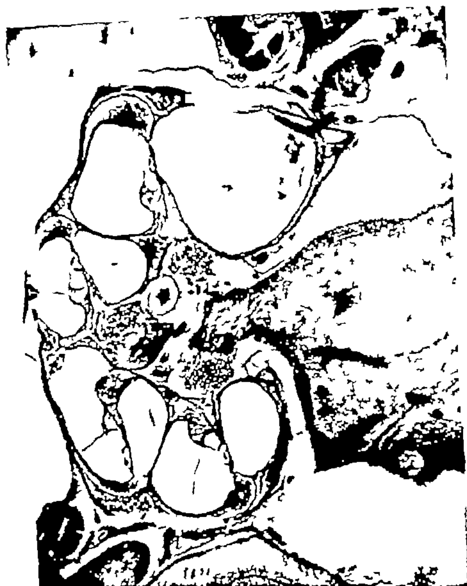


Fig. 7 The cochlea, the VIIIth nerv and the cochl ar uclei. 40

parts of the nuclei are seen in square sections in the Figs. 10 and 11 Fig 10 shows the dorsal (top of picture) and the posteroventral nucleus, and Fig 11 the anteroventral (top) and the posteroventral nucleus.

The number of nerve cells counted, compared to ther species are seen in Table 2.

The dorsal cochlear nucleus was counted separately and the reason for this was our present interest in the role of this nucleus (Hall, 1964 Osen & Jansen, 1965) in different pecies (see Discussion)

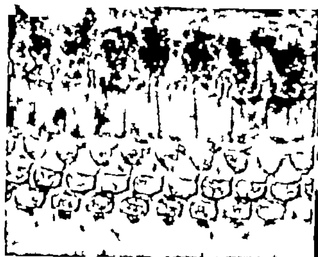


Fig 5

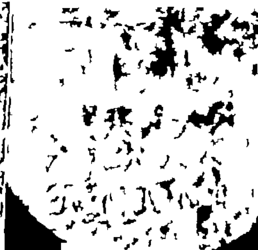


Fig 6

Fig. 5 Th outlin of the three rows of inner hair cells, a d the single row f outer hair cells. × 1000

Fig 6 Th W" formed by th tip of the h irs on top f the hair cells. 1250

The spiral ganglion situated in the modiolus, contain great oval cells, with their nuclei centrally placed The space separating this ganglion from the cochlear nuclei is narrow (Fig 7) which was the reason why a preparation of this short VIIIth nerve for a fiber count, was not successful

Fig 8 shows the usual square section of the organ of Corti where especially the lamina spiralis ossea secundaria is remarkable (Reysenbach de Haan 1958)

The cochlear nuclei are situated in the lateral part of the medulla at the lateral corners of the fossa rhomboidea. They bulge out like an extramedullary pouch on each side, occupying a substantial part of the brain stem

The following facts about the cochlear nuclei were determined The complex of the cochlear nuclei measured 2 mm in length on average, forming a spindle, whose diameter in its widest part was 0.5 mm The cubic content was 0 6 mm^3 which represents one fourth of that in the cat

The cochlear nuclei can be divided into three main sections, as in cat and man the anteroventral, the posteroventral and the dorsal nucleus (Fig 9) Fig 9 shows drawings of square sections of the cochlear nuclei which is used for computing the volume and the number of nerve cells The nerve enters rostroventrally and the nerve root continues dorsocaudally between the antero- and the posteroventral nucleus.

The volume of the cochlear nuclei are seen in Table 1 compared to other species (Hall 1964 and 1967) It is seen in this table, that the cochlear nuclei in bats are far more voluminous than in the others, compared to the size of the animals They constitute about one third of those in a kitten, and one sixteenth of those in man which is a substantial magnitude for an animal weighing only 10–12 g Representative pictures of the three different



Fig. 10.



Fig. 11

Fig. 10. The dorsal and the posteroventral nucleus. 240. Separation line stippled.
Fig. 11. The anteroventral and the posteroventral nucleus. 240. Separation line stippled.

The cochlear nucleus in the bat contains on average 40 000 cells, whereof 7000 cells belong to the dorsal cochlear nucleus. The layer of granular cells covering the cochlear nucleus (especially the dorsal one) was not counted, only the nerve cells containing a nucleus surrounded by a distinct cytoplasm.

As it is of great interest to know also the ramification of the nerve fibres distributed upon the cells of the cochlear nuclei, this point was studied separately.

A saggital section, showing the ramifications of the nerve inside the nuclei is shown in Fig. 12. (Osen, 1966) The ascending fibres, to the left in the

Table 1. *Volume of the cochlear nuclei (in mm^3)*

Bat	Kitten	Homo	Porpoise	Fin whale	White whale
0.6	2	10	60–100	205	320



Fig. 8. Th

THE COCHLEAR NUCLEI IN TH

Fig. 9 Schematic drawing f sq re s
drawn



FIG. 10



FIG. 11

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Fig 8. The organ of Corti

THE COCHLEAR NUCLEI IN THE BAT PLECOTUS AURITUS

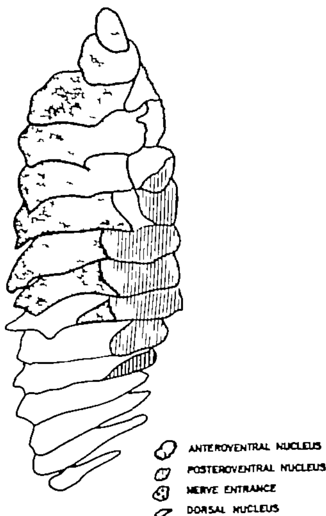


Fig 9 Sch m tl drawi g f square secti n f th cochl ar n l i. Ea h 10th lide i drawn

DISCUSSION

The outer ears in bats are extremely well developed, and also their middle and inner ears are of considerable size compared to the size of the animal. As to the general anatomical structure however this is similar to that of other mammals. The outer ears, measuring more than $^{1}/_{3}$ of the body and the ear-drums, whose size is about $^{1}/$ of those in man, show that we are dealing with a highly specialized system. The cochlea also has considerable dimensions, but the most remarkable feature was the size of the cochlear nuclei. They were not only voluminous, but also contained 40 000 nerve cells, which is about 40% of the number of nerve cells in man. As their whole existence is based upon echolocation and they possess no obvious communication, all these cells must be mainly employed in the perceiving of echoes. 7000 or about 17% of the cells were situated in the dorsal cochlear nucleus. Comparing man, cat and different whale species it is seen, that in species which apply high frequencies or supersonics for the finding of prey echolocation or perhaps communication (whales) the number of nerve cells in the dorsal cochlear nucleus is diminishing, while in man and kitten this part occupies a substantial part of the complex. Thus it is reasonable to assume, that the transmission of the high frequencies in the echolocating species is more specifically carried out by the ventral nucleus, which is the dominating one, especially in whales, but also in bats, both concerning the volume and the number of nerve cells. The dorsal cochlear nucleus, however is rudimentary in whales, but somewhat more pronounced in the bats. In the dorsal cochlear nucleus the cells are arranged in rows or layers, somewhat like a cortical arrangement, but this is not so prominent as in cat or man. The nerve fibres reach the cells of the dorsal nucleus through the posterior part of the posteroventral nucleus (Fig. 12) where they are twisted 180 (Osen, 1966) This point provides the anatomical basis for the findings of Rose *et al.* (1959) who have shown that the low frequency carrying fibres are situated ventrally and the high-frequency carrying fibres dorsally in each part of the complex. To achieve this the fibres converge when passing through the posteroventral nucleus, and then twist 180

This study of structures and measurements in bats has revealed large dimensions both of the cochlea and of the cochlear nuclei compared to other species. Also the complexity of the cochlear nuclei in the bats points to a highly developed system supervising their supersonic abilities.

ZUSAMMENFASSUNG

Das äussere und innere Ohr der Fledermäuse *Plecotus Auritus*, sowie di Cochleariskern derselben werden beschrieben. Der Verfasser hat 20 Exemplare gesammelt, in welchen die Cochlea von 10 Exemplaren nach der Methode von



FIG. 12. Th ramifications of th VIIIth nerve in id the cochl a l l 120

picture, spread out fanlike in the anteroventral nucleus. The descending ones however converge in the caudal part of the posteroventral nucleus where they are twisted 180 degrees, and then also these spread out fan like.

TABLE 2 *Number of nerve cells in the cochlear nuclei of different species*

Species	N f cell T t l	Dors. nucl.	Dorsal L f total
Bat	40,000	7,000	17
Kitten	100 000	29 000	29
Homo	96,000	30 000	32
Porpoise	600,000	40,000	6.6
Whit whale	1 650,000	24,000	1.5

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SOME EXPERIENCES WITH THREE HUNDRED STAPEDECTOMISED PATIENTS

L. E. Floberg, B. Ivstam and T. Lundborg

From the E.N.T. Clinic and Audiological Unit, Södersjukhuset and Hörselkliniken, Stockholm, Sweden

At the E.N.T. clinic, Södersjukhuset, stapedectomy (with vein graft in the oval window and application of polyethylene tube on incus) has become the method of choice for 500 operated otosclerosis patients 1962–1967. The results from 300 consecutive stapedectomies 1962–1967 are discussed. The experience of the actual case material reveals that, for instance, the age of patients at admission is immaterial and that many audiometrically classified C- and D-cases seem to have very good prognostic possibilities. The postoperative hearing results—according to the definition of closed gap and average improvement in the middle frequencies—are good, ca. 93% success as in many other series. Postoperative deafness has appeared in 4 cases out of 300. From the point of view of hearing rehabilitation the results have been especially satisfactory: 90% of the patients could dispense their hearing aids or change from bodyborne to headborne apparatus, thus this factor is the essential indication for an eventual operation. There is needed a continuous postoperative hearing control, especially concerning "late" cochlear lesion. Uniform definitions and classifications of case materials, for instance common use of a A.D.P.-form, will probably give a more certain basis for prognostic evaluation and possibly indicate changes in operative technique.

During the period 1962–1967 more than 500 otosclerosis patients have been operated upon in the E.N.T. clinic of Södersjukhuset. In the majority of cases stapedectomy has been performed with vein graft in the oval window niche and application of polyethylene tube on processus longus incudis. During the whole period the original Rosen-technique has never been used, nor has stapediolysis been performed directly on the footplate except in 3 cases with very advanced niche otosclerosis. On the other hand, Fowlers technique i.e. stapediolysis with anterior crurotomy and partial stapedectomy has been used in quite a number of cases. Gaining experience that this kind of technique too often causes reankylosis—which is well known—this type of operation has been abandoned gradually. It has been performed only in cases with "ideal circumstances" i.e. mostly with restricted otosclerotic areas around the anterior crus, which can be easily removed, with the rest of the footplate freely movable, with intact incudo-stapedial

H Engström präpariert sind, von den letzteren 10 sind die Cochleariskerne nach der von dem Verfasser in 1964 beschriebenen Methode präpariert worden weiter sind ihre Volumen sowie ihre Nerven Zellenanzahl berechnet, und die Verteilung der primären Fibern wird beschrieben

Die Verhältnisse dieser Bildungen in der Plecotus Auritus werden mit anderen Arten (Katzen Walen und Homo) verglichen

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R i d August 30 1968

Symptomatology

The clinical facts have been registered on special audiology journals suited for automatic data processing (Lundborg, 1965) and the actual study has been made possible by grants from "Sjura". In this connection only a relevant part of all information, which is available from the data processing, is presented.

Distribution of Age

The actual patient material merits special interest from this point of view according to Table 1. If one compares this condition with the indication for fenestration, where an age above the middle age was considered *a contra-indicative factor*. The distribution of age *among* the 300 stapedectomised patients reveals first a rather higher average than among fenestrated patients (Skoog, 1953) and also, that a relatively large group of patients—31—is more than 70 years old, in two cases even 80 years old, which demonstrates that a "maximum age" does not seem to exist, which also has been demonstrated by Andersen & Warrer (1968) and others. When the case material is classified in A B-C and D-cases (according to Shambaugh) there is another interesting aspect to compare with for instance Skoog's (1953) material of fenestrated patients (Table 4).

In a rather large group of the patients the average of A.C.-level in the middle frequencies was less than 80 dB, in some cases even at the 100 dB level or without any registrable A.C. in certain frequencies. These patients were almost, without exception, old hearing aid cases, where at earlier evaluation indication of fenestration was not considered to exist. In many of these cases there had also been made attempts earlier to perform indirect stapediolysis.

As can be seen from Table 4 the B.C. levels of the case material revealed that almost / of the cases were *D-cases* with audiometrically established *cochlear lesion with mean of B.C. level less than 35 dB, in some cases even* without registrable B.C.-level. Most of the patients were *C-cases, a minor* group were B- and A-cases.

A.D.P.-registration has used 10 dB steps, why a certain evaluation is necessary when the patients shall be grouped according to Shambough. It is, in any case, obvious that on the whole in the actual case material, there is a clear tendency to an average of impaired cochlear function compared with Skoog's (1953) *material of fenestration cases*. In cases with clearly impaired cochlear function or in cases where this function is very difficult to evaluate the performance has naturally been done *as an attempt to get some* utilization of the least usable ear often on the patient's *eager request*. Another diagnostic difficulty encountered in such cases, is to evaluate *if* and *how* the cochlear lesion is progredient. When it has been possible to repeat the examinations on the same patient over a fairly long period and

joint and with good subjective improvement of hearing This changed attitude towards the partial stapedectomy technique is apparent for instance from the fact that during 1962 44 stapediolysis were performed, but during 1966 only 3 Thus stapedectomy has become the method of choice and stapediolysis with partial stapedectomy has—as mentioned—been performed *only exceptionally under* special circumstances. Therefore there is little reason to discuss results and prognosis in stapediolysis, because these facts are well known Because the patients in a large group of the cases were fairly aged and using hearing aids for many years, and a modified type of polyethylene tube has been used there are special reasons—primarily sociomedical—to discuss *the results after stapedectomy in 300 consecutive cases.*

The anatomical and histological conditions in the oval window niche with all variations of the otosclerotic processes etc. are well known thus there is no reason to present details in this connection

Diagnosis

As a rule the patients have been examined with a test battery comprising pure tone-audiometry speech-audiometry Békésy audiometry (Grason Stadler Narrow Band Noise, mod E 800) Gellé-test and registration of *stapedial reflex (Madsen model Z061) The examinations with this test* battery give a well known result pattern

(*a*) Gap between A C and B C with types of curve in A C. mostly revealing rising, flat or gradual downward curves In a few cases other curve types (Table 3) and with B.C. in varying levels (Table 4) i e corresponding to A B-C and D-cases according to Shambaugh s terminology

(*b*) Discrimination scores corresponding to B C. level

(*c*) Békésy audiograms revealing type I Jerger

(*d*) Gellé-test negative i e revealing only direct or "osseal bone conduction but not "indirect" or osseotympanal" bone conduction

(e) Stapedial reflexes cannot be registered. Only in one case has there been registered normal stapedial reflexes, which—after double check—could be explained at operation by the presence of very thin stapedial crurae in connection with a totally fixed foot plate thus making possible contractions of the stapedial muscle

Several tests have been used on each patient with the aim of getting consistent results Practically this routine has been verified by a correct diagnosis in all cases The evaluation of bone conduction level and cochlear function—as is well known—often reveals difficulties and uncertainty especially in the group of D-cases, for instance in old patients having tinnitus and using an *unilateral hearing aid for many years During these conditions* the ear actual for operation at examination may offer special difficulties because of its lack of daily life stimulation

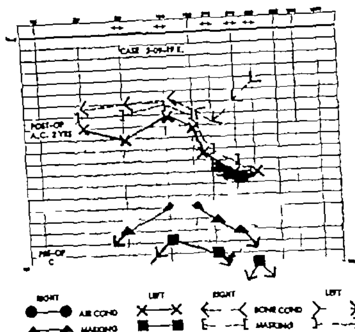


Fig. 2. Case 1912–09–19. E. K. Male patient with bodyborne hearing aid with ry insufficient communicati od social condition (alcoholic). *Post.op.: using headborne hearing aid* ly occasionally; after one year well rehabilitated and socially cooperating.

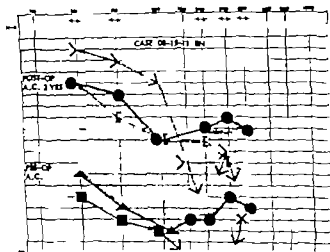


Fig. 3. Case 1908–12–11. E. M. Female patient, foreign born, using body-borne hearing aid, not Swedish-speaking after 10 years. Post.op.: headborne hearing aid occasionally. Good linguistic progress.

in the oval window niche, and at a slight pressure on malleus, smoothly following the movements of incus. This is a pre-requisite to get adequate columellar contact with permanent fixation of the prosthesis on incus but without pressure on the long process. It is practically impossible for a

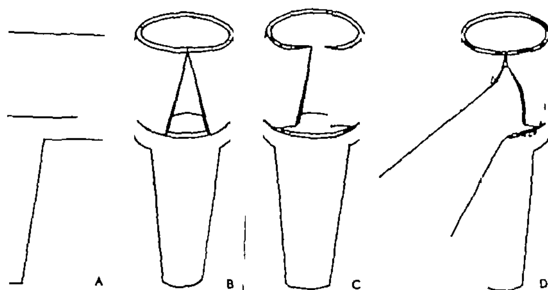


Fig. 1. The polyethylene stapes prosthesis. (*A*) The "collar" of prosthesis is formed by transversal section of the circumference. (*B*) oblique longitudinal incision in the "collar". (*C*) with the prosthesis introducer in position, the longitudinal opening is widened enough to grip around incudal process. (*D*) after removal of introducer proc. lenticularis incudis remains in a smooth "self locking position".

when the B.C. level and discrimination scores have revealed successively deteriorated cochlear function, the performance has been refused. In some cases of this type, without any operative performance a total cochlear lesion has been stated. If some of these cases had been operated upon, with all probability the surgical performance would have been considered responsible for the cochlear lesion. There is reason to believe, that this factor may partly exist in many case materials, but—as far as we are aware—it has not been sufficiently emphasized in the discussion of postoperative cochlear lesions. When the case material has been checked over a sequence of years, it may be possible to get better statistically based rules concerning the relation between audiometrically established cochlear reserve and prognosis. With actual experience, in isolated cases, it seems very difficult to formulate universal prognostic rules.

The stapedectomy performance was a modified Goodhill technique (Goodhill 1961) i.e. removal of the foot plate with a vein graft in the oval window niche and application of a polyethylene tube on processus longus incudis. The prosthesis was cut to suitable length and shape according to Fig. 1. To ensure good fixation of the prosthesis to the incudal process without causing simultaneous circulatory disturbance of the process, the prosthesis was shaped to be "self locking". According to actual experience these requirements were fulfilled with the type used. The longitudinal oblique incision of the prosthesis collar formed an opening with two wings clutching the incudal process as if in a grip. When the introducer was removed the polyethylene tube was placed in position firmly on incus, well centred

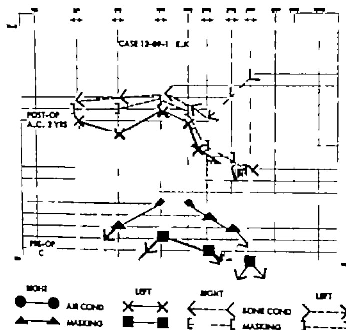


Fig. 2. Case 1012-09-10. E. K. Male patient with bodyborne hearing aid with ry insufficient communicati and social condition (alcoholic). Post.op., using headborne hearing id only occasionally; after one y well rehabilitated and socially cooperati g.

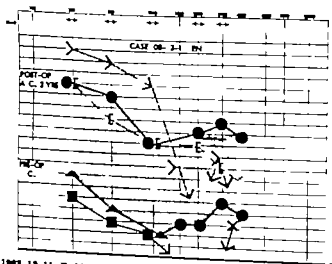


Fig. 3. Case 1008-12-11. E. M. Female patient, foreign born, sing body borne he ring id, not S edish-speaking after 10 years. Post.op. headborne hearing aid occasionally. Good li guistic progress.

In the oval window niche, and at a slight pressure on malleus, smoothly following the movements f incus. This is a pre-requisit to get adequate columellar contact with permanent fixation of the prosthesis on incus but without pressure on the long process. It is practically impossible for a

TABLE 1 *Distribution of age in 300 stapedectomised patients*

Age yrs	No. of pts.
> 70	51
60–69	91
51–59	84
41–49	50
< 40	24
Total	300

prosthesis of this type correctly applied on the incudal process, to be dislodged In our experience this type of tube has never failed in this connection (Siirala, 1965) Gaining good experience with this type of technique we have not found it worthwhile to use any other form of technique (Portmann technique, teflon prosthesis, Schuknecht prosthesis etc.) The idea has principally been—as Portmann (1965) stated at the VIII Int. congress of O R.L.—"to achieve a permanent opening in the oval window and create a columellar contact between incus and the oval window How this is performed in detail is a secondary question" The details in operative technique are well known and need thus not be discussed. Local anesthesia was normally used In only 10 cases was general anesthesia performed with intubation The oval window opening was covered with a vein graft from the dorsal part of the hand gelfoam or other foreign material was not used

Results

There have been no complications in fom of otitis, labyrinthitis or meningitis in direct connection with operation, which has been related in many other case materials On the other hand, in several cases there have

TABLE 2 *Air conduction level in 300 stapedectomised patients*

Mean of 500–1000–2000 Hz.

A.C. level, dB	No. of pts.
> 80	41
71–80	44
61–70	67
51–60	91
41–50	48
40	9
Total	300

TABLE 3 *Type of curve in 300 stapedectomised patients*

Type of curve	No of pts.
Flat	145
Rising	75
Gradual down	53
Marked down	9
Trough	5
Humpback	1
Others	1
No registrabl A.C.	8
T tal	300

TABLE 4. *Preoperative bone conduction level in 300 stapedectomised patients*

B.C.-level, dB	No. of pts.
Not reg.	8
>50	19
50–41	40
40–31	84
30–21	113
20–11	34
<10	2
Total	300

been nose and throat infection shortly after the performance—tonsillitis, sinusitis and in one case also an acute otitis with spontaneous drum rupture—but in no case with simultaneous lesion of the labyrinth. According to our experience the vein graft put in proper position in the oval window niche, thus ensures a good protection of the labyrinth. Special complications: temporary facial paresis four cases, liquor drainage in one case, which healed without cochlear lesion.

Hearing Results

The great majority of patients enjoy exellent hearing postoperatively as is well known from many other case materials. To evaluate the improvement of hearing and the effect of operation, a follow up study for several years is necessary. It is obvious, however, that the actual case material reveals a clear tendency concerning good hearing results, thus there seems to be no reason to change the technique used at the moment. The follow up of the cochlear function should decide whether a less radical technique is needed.

TABLE 5. *Postoperative hearing results*

	Patients examined		
3 months	300	Closed air-bone gap	284
		Air-bone gap	8
		Deaf	4
		Missed*	4
1 year	292	Closed air-bone gap	283
		Air-bone gap	9
2 years	232	Closed air-bone gap	222
		Air-bone gap	10

* Two patients deceased by irrelevant disorders, two patients moved away to unknown addresses. The three month control revealed closed air bone gap in all four cases.

From the *hearing rehabilitation* point of view there have been such satisfactory results (i e that the patient not need to use a hearing aid or only has to utilize a hearing aid occasionally or can use a headborne hearing aid instead of a bodyborne apparatus) that this positive experience now by the authors—as for instance by Johansen (1967)—*is looked upon as the essential operative indication* Because of this a preliminary report would appear useful

The registration of the postoperative A C and B C levels has followed Goodhill's system (Goodhill 1961) i e firstly to note if there is an air bone gap or not. In the cases without air–bone gap the postoperative average improvement of hearing in the middle frequencies (500–1000–2000 Hz) varied between 25–30 dB and 55–60 dB Why an operative elimination of a totally fixed foot plate results so differently may be assumed to depend on the partly unknown factors which determine the variations of Carhart notch

More than 50% of the patients (152 of 300) had a preoperative A.C. level—mean of middle frequencies—less than 60 dB and therefore needed a bodyborne hearing aid permanently The other 148 patients mostly used a hearing aid or had a hearing aid prescription at the time of operation. The postoperative hearing gain thus made it possible for 90% of the patients to dispense with the hearing aid used for many years or change from a bodyborne to a headborne apparatus. This mode of amplification for most patients is far preferable thus making it possible, for instance not to stop at call which of course means great relief and benefit (Table 2)

In this connection some of the foreignborn patients merit special interest from the rehabilitation point of view In four cases (all women) who permanently used bodyborne hearing aids, one could state that the degree *of hearing impairment* (A C.-curves in the 70–80 dB level) despite amplification *did not permit the learning of Swedish*, though there was a linguistic exposition for more than 10 years The postoperative hearing gain—with use of headborne hearing aids occasionally—resulted in social hearing rehabilitation including adequate linguistic progress (Fig 3) There are apparently certain hearing psychologic factors that constitute an adequate *sound stimulation needed for linguistic learning*

Cochlea-complications (dead cochlea) appeared in 4 cases in 3 patients in immediate connection with the operation One patient primarily had a good hearing improvement, though not a closed air–bone gap but at the 3 month control the preoperative gap was again established. A revision was *proposed* but the patient did not return until the 12 month control and showed then complete deafness on the operated ear Tympanotomy revealed that the oval window was completely filled with the vein graft Normal looking middle ear mucosa covered the tube, which remained firmly in normal position on incus, which only had a very slight groove at the circumference of application but without any sign of necrosis. Thus there were no macroscopic findings that could explain the cochlear lesion The post

operative cochlear lesions following stapes surgery vary in different case materials according to Beickert s exposé (Beickert, 1963) in Berendes–Link–Zöllners Handbuch, which are based on ca 1000 references, there is an aver age cochlear lesion rate of 4%.

The postoperative impairment of B.C.-level, especially in 2000 and 4000 Hz has often been discussed. In 139 consecutive cases this factor was con trolled 12 months after operation, which at 2000 Hz revealed improved B.C.-level (between 5–30 dB) in 108 cases, status quo in 23 cases and a slight impairment (5–15 dB) in 8 cases. The corresponding figures at 4000 Hz were improvement 57 status quo 50 impairment 28 (5–10 dB) and 4 (15–30 dB) The B.C.-levels were also controlled in 61 consecutive cases 2 years after operation. At the 2000 Hz-frequency 47 revealed improvement (between 5–30 dB) 11 status quo and 3 had impaired levels (10–15 dB) At the 4000 Hz frequency improvement in 21 cases status quo in 19 a slight deterioration (5–10 dB) in 15 and in 6 cases an impairment between 15–25 dB The 6 cases revealing 15–25 dB impairment at 4000 Hz 2 years after operation and the corresponding 4 cases from the one year control were patients of varying age (born between 1891–1932) and had principally all fl t A.C.-curve types. The age factor seems difficult to evaluate, but worth mentioning is that among these patients there is nobody with a gradual down curve type which statistically should be expected. Therefore there is reason to check postoperative B.C.-levels over a sequence of years, which perhaps may reveal some clue as to which factors constitute these partial cochlear deteriorations.

CONCLUSIONS

The experiences gained in the actual case series have on the whole been ery positi e from the hearing rehabilitation point of view especially con cerning the possibilities to perate and rehabilitate many "border line cases for instance old patients using bodyborne hearing aids for many years with audiometrically established severe cochlear lesions. It may often be difficult to compare postoperative hearing results from different publications, because of their different characters from so many aspects (the con stitution of A B-C- and D-cases etc.) A more adequate comparison can be made between some different case materials when a group of cooperating clinics re using the same definitions and evaluations with registration on the same A.D.P.-audiology journals. A team in the Swedish Otolaryng. Society is running such a project.

ZUSAMMENFASSUNG

An der O-N-H Klinik des Sodersjukhuset in Stockholm ist in dem Zeitraum von 1962 1967 an einem Material von insgesamt etwa 500 Otosklerose-Patienten neben anderen Operationsmethoden wahlweise die Stapedektomie, d. h. Abdeckung des ovalen Fensters mit Vene und I terposition eines Polyäthylenröhrchens als

Stapes-Ersatz, durchgeführt worden Wie das aktuelle Material von 300 stapedektomierten Patienten erkennen lässt, ist das Alter des Patienten nicht von wesentlicher Bedeutung. Die postoperativen Hörgewinne, die aus dem Ausgleich des Schalleitungsdefizits und der durchschnittlichen Schwellenwertsverbesserung im mittleren Frequenzbereich zu entnehmen sind, sind in guter Übereinstimmung mit den Erfahrungen von vielen anderen Autoren In dem vorliegenden Material ist besonders hervorzuheben dass viele Patienten, die auf Grund des audiometrischen Befundes als C- bzw D-Fälle einzustufen sind gute Operationsresultate gezeigt haben und damit scheinen auch für diese Gruppen die prognostischen Aussichten recht gut zu sein Postoperative Innenohrertaubungen traten nur bei 4 Patienten von insgesamt 300 ein

Mit Hinblick speziell auf die Rehabilitierung der stapedektomierten Patienten war das Resultat im ganzen gesehen sehr günstig 90% der Patienten konnten entweder ihr Hörgerät gänzlich ablegen oder aber an Stelle des bisherigen Taschengerätes zu einem Hinter-dem-Ohr-Gerät übergehen. Dies ist zweifelsohne ein sehr wesentlicher Gesichtspunkt in der Indikation zu einer Operation.

Ein wesentliches Erfordernis sind kontinuierliche postoperative Hörkontrollen speziell zur Feststellung von eventuell später eintretenden Innenohrhörverlusten

Abschliessend wird vorgeschlagen bei der Präsentation der verschiedenen Materialzusammenstellungen von einheitlichen Definitionen und Klassifizierungen auszugehen z B durch allgemeinen Gebrauch der A.D.P Journale wodurch sich wahrscheinlich eine bessere Ausgangsbasis für die prognostische Beurteilung erreichen liesse, was in der Folge eventuell zu einer minder radikalen Operationstechnik führen kann

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ATYPICAL CILIA IN NORMAL HUMAN AND GUINEA PIG MIDDLE EAR MUCOSA

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Normal middle ear mucosa from human a d guinea pig material was prepared for electron microscopi studies. Unusual cilia which contain two or more xial filame t complexes were occasionally encountered in these preparations. Detailed observati ns n the fine structure f these cilia ha e been presented, discussed and compared with ordinary cilia.

The mucosa of the middle ear and the eustachian tube is continuous with the mucous membrane of the nasopharynx. The lining of the middle ear cavity is also an extension and modification of respiratory mucosa Cells with cili in the middle ear of human are morphologically identical to those of the respiratory system (Kawabata & Paparella, in preparation)

In examining the ultrastructure of the normal middle ear mucosa of human and guinea pig, we observed cilia with unusual internal structures. This paper describes these cilia in detail. Several observations concerning unusual cilia in pathological conditions have been described (Duncan & Ramsey 1965 Galto *et al* 1965)

MATERIAL AND METHODS

A specimen of middle ear mucosa was surgically obtained from a patient with conductive deafness resulting from head trauma. This patient had no history of inflammatory ear disease. This specimen was fixed with 1% osmium tetroxide in veronal buffer ph. 7.4 The middle ear mucosa from an adult male guinea pig was fixed with 3.5% glutaraldehyde in phosphate buffer ph. 7.4 and the specimen was postfixed in 1% osmium tetroxide and then dehydrated and embedded in Epon 812. Ultrathin sections were cut with glass knives on a LKB microtome, double stained with uranyl acetate and lead citrate, and observed in an RCA EMU-3G electron microscope

RESULTS AND DISCUSSION

In recent years electron microscopists have reviewed in detail the complex internal structure of cilia and flagella (Fawcett, 1954 Lansing & Lamy

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1961 Rhodin 1960 Rhodin & Dalhamn 1956 Roth & Shigenaka, 1964) All cilia contain a constant number of eleven internal fibrils, two in the center and nine evenly spaced around them This pattern is amazingly constant throughout the plant and animal kingdoms (Fawcett, 1961) Rhodin (1966) described the ultrastructure of ciliated cells of human tracheal epithelium. No obvious differences were found between the general morphology of cilia in human middle ear mucosa and the tracheal epithelium.

However cilia containing an unusual arrangement of internal structure were occasionally encountered in both human and guinea pig middle ear mucosa The internal structure of the cilium is termed by Fawcett (1961) the Axial Filament Complex It consists of the two central fibers and the nine peripheral fibers mentioned above. In cross section the central fibers are circular and separated from each other They have a dense surface layer with a less dense interior which gives them a tubular appearance. The outer fibers in cross section are oblong with the short axis running radially and the long axis is divided into halves by a radially oriented septum, which is continuous with the dense surface layer of the fiber and is of the same thickness (Fig 4) The peripheral fibers have a "double-barreled or figure eight (Fawcett 1961) appearance in cross section, whereas the central single fibers have a simple circular pattern This principal pattern, axial filament complex, is usually enveloped by a ciliary membrane which is continuous with the free surface of the ciliated cell The cilia illustrated in this paper have anomalous internal structures with two or more axial filament complexes in a common ciliary membrane. Its fine structure however appears normal

FIG. 1 Electron mi rograph f guinea pig middl r mucosa showing profil of on ntire u usual llium (*arrow*) *One of th axial filament compl es is directly co nected* with th basal body (*B*) but the oth i not found in the ph t graph, pre umably du to secti ning directi n *C* L ual cilium *M* mitoch nd ia. 28,000

FIG. 2. Electron micrograph of middle ear mucosa f n rm l gui pig showing the cross section of cili Besides the n rm l illa (*C*) th unu l illum (*a w*) which ntain two pairs f axi l fil ment complexes and el ped in the n rm l ciliary membrane can be The ntire ppe rance f thi cilium i v l in h pe 25,000.

FIG. 3 Electron micrograph f n rmal middl ar mucosa of human h w g cilia in ros ecti n There re two kinds f unu ual cilia, on (*ngl ar w*) i th sam that seen n Fig 2, the th (*d ubl arr w*) tai three pairs of ial filam nt compl x The shape of the latt ppears triangul *C* N rmal ili 20,300

FIG. 4 Electron micrograph of an unusu l llium (*a row*) f gui a pig middle mu osa In the cro secti n of this llium which i co red by i gl il ary m m brane there are three pairs f ce tral fibers, how the circumf rential peripheral fibers c rrespo di g t the central o es are n t tirel represe ted. Th o i t ti n of each central pair i parallel, a d this directi is, furtherm re parall l to that f surrounding n rmal cil 25,000


C
C
C
C
C
1
2
3
4

1961 Rhodin, 1959 Rhodin & Dalhamn 1956 Roth & Shigenaka, 1964) All cilia contain a constant number of eleven internal fibrils, two in the center and nine evenly spaced around them This pattern is amazingly constant throughout the plant and animal kingdoms (Fawcett, 1961) Rhodin (1966) described the ultrastructure of ciliated cells of human tracheal epithelium No obvious differences were found between the general morphology of cilia in human middle ear mucosa and the tracheal epithelium.

However cilia containing an unusual arrangement of internal structure were occasionally encountered in both human and guinea pig middle ear mucosa The internal structure of the cilium is termed by Fawcett (1961) the Axial Filament Complex. It consists of the two central fibers and the nine peripheral fibers mentioned above. In cross section the central fibers are circular and separated from each other They have a dense surface layer with a less dense interior which gives them a tubular appearance The outer fibers in cross section are oblong with the short axis running radially and the long axis is divided into halves by a radially oriented septum, which is continuous with the dense surface layer of the fiber and is of the same thickness (Fig 4) The peripheral fibers have a "double-barreled" or "figure eight (Fawcett 1961) appearance in cross section, whereas the central single fibers have a simple circular pattern This principal pattern, axial filament complex, is usually enveloped by a ciliary membrane which is continuous with the free surface of the ciliated cell The cilia illustrated in this paper have anomalous internal structures with two or more axial filament complexes in a common ciliary membrane Its fine structure however appears normal

FIG. 1 Electron micrograph of guinea pig middle ear mucosa showing profile f one ntire unusual cilium (*arrow*) On of the axial fil ment mpl xes is directly connected with the basal body (*B*) but the othe I not f und in the ph tograph, presumably due to sectioning directl n. *C* L u l cilium *M* mitoch ndri 28,000

FIG. 2 Electron micrograph of middl a mu os f n rmal guin a pig bowi g the cross secti n f cilia Be id s th normal cilia (*C*) the u usu l cilium (*arrow*) which contains two pairs of axial filam nt complexes nd eloped in the n rmal ciliary membran can be se n. The entire appe rance of this cili m is l in shape 25,000

FIG. 3 Electron micrograph of n rmal middl ar mucosa of human sh wi g cili in ross secti ns There re two kind of unu ual lia, ne (*ingl ar w*) is the same s that seen in Fig 2, the ther (*d bl row*) contain three pairs f xi l fil m t complexes The shape f the l tt appears t langul *C* N mal ilia 20,300

FIG. 4 El ctron micrograph f n unu ual ilium (*arrow*) of guine pig middl muco a. In th er secti n f thi ilium which co red by l gl liary m m br n th re ar three p irs of ce tral f bers, howev the circ mf renti l peripheral fibers corresponding to the central ones re not ntirely repr se ted The orient ti n f e ch ce tral pair is pa ll l, and thi directi is, furthermore parall l to that of surrounding normal cil 23,000

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Some cilia are doubled having two axial filament complexes enveloped by a completely continuous membrane (Figs 2 and 3) Compared with typical cilia around it, the axial filament complex, fine structure shape and size are identical The appearance of this cilium is oval or oblong in shape Infrequently a cilium has three axial filament complexes whose fine structure appears normal The shape of this cilium is triangular compared with the oval shape of the double cilia (Fig 3)

Furthermore multicilia which have three pairs of central fibers are infrequently seen, but they are not always completely surrounded by paired peripheral fibers (Fig 4) That this phenomenon is due to artifact remains a possibility However this does not seem likely because the axial filament complexes are enveloped within a continuous ciliary membrane and the fine structure seems to be well preserved

Fig 1 demonstrates the profile of a double cilium The right and middle cilia in the photograph (Fig 1 *C*) have an ordinary profile of axial filament complex, but the left cilium has two pairs of complexes within a single ciliary membrane The axial filament complex terminates at its base in a basal body at the level of the cell surface (Fawcett 1961 Duvall *et al* 1966 Gaito *et al* 1965 Gibbons, 1961 Rhodin & Dalhamn 1956) In the double cilium one basal body continuous with the complex is demonstrated The second basal body of the complex does not appear in the photograph presumably due to the angle of sectioning

Coordinated movement of cilia is not well understood at the present time However several observations suggested a method of discerning the direction of the effective stroke of the cilium (Fawcett & Porter 1954 Duvall *et al* 1966) The orientation of the central paired filaments is the same in all of the cross sections. Lines drawn through their central axis are parallel, and the direction of the ciliary beat is perpendicular to these lines according to Fawcett & Porter (1954) The orientation of the central filaments in the unusual cilia in our material is parallel to the central filament of typical cilia appearing around them This arrangement is of interest, but the functional significance if any of the unusual cilia is unknown.

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ZUSAMMENFASSUNG

Die normale Mittelohrschleimhaut in Menschen und in Meerschweinchen wurden mit dem Elektronenmikroskop untersucht. Ungewohnliche Zilien, die zwei oder mehrere „axial filament complexes" enthalten wurden in diesen Präparaten gefunden Die ultrastrukturellen, morphologischen Einzelheiten dieser ungewöhnlichen Zilien wurden mit den normalen Zilien verglichen und diskutiert

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OBLITERATION OF THE CAVITY IN MASTOIDECTOMY

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A modification of Rambo's temporal-musculoplasty for obliterating the cavity following mastoidectomy is described and the early and late operative results in 110 cases of active chronic otitis are presented. The use of retro-auricular tissue together with the temporal muscle gives a long, cranially pedicled flap one end of which is placed into the antrum and the other towards the tip of the mastoid process. To avoid stenosis, the skin of the auditory canal and of the external meatus is incised longitudinally By this method more than 90% of the ears dried, and there were only a few postoperative complications. The late results showed that the ears remained dry and that there was no recurrence of cholesteatoma or of infection The results in respect to hearing are briefly mentioned

Even in the case of a perfectly performed mastoidectomy the patient as well as the surgeon often has great difficulty in keeping the radical cavity dry Foul smelling detritus or crusts may accumulate there, and in some cases the cavity has to be cleansed at intervals

For many years, therefore, otologic surgeons have tried to diminish or obliterate the cavity by means of free or pedicled grafts. Free grafts have been of bone (Schiller & Singer 1960) cartilage (Guilford, 1960) or muscle and fascia (Heermann 1962)

Pedicled grafts are made of the tissue surrounding the cavity temporal muscle based superiorly (Rambo, 1958) or posteriorly (Thomas, 1963) the retro-auricular subcutaneous tissue together with the neck muscles (splenius capitis, sternocleidomastoid) based either superiorly or inferiorly (Guilford, 1961 Elbrønd, 1963)

Palva (1963) used the retro-auricular subcutaneous tissue with periosteum and the retro-auricular muscle for a pedicled graft with a broad base ventrally Since the flap is usually too small Palva inserted gelfoam between the flap and the bone Instead of inserting gelfoam Svane-Knudsen (1967) preferred leaving a small cavity at the site of the antrum and attic

Thorburn (1963) used the bipedicled temporal muscle one pedicle supplying fascia to cover the middle ear and the other one to obliterate the cavity

The introduction of pedicle grafts for obliterating the cavity is definitely one of the great advances in otologic surgery It fulfills several demands at the same time (1) Obliteration or diminution of the cavity reducing

the retention of detritus to a minimum (2) *creation of an amply vascular* ized layer of tissue in the cavity which will thereby be more easily and more rapidly epithelialized (3) *restoration of approximately the same anatomi cal status* in the auditory canal as prior to the operation. This is of particu lar importance when tympanoplasty is done in the same stage as mastoidec tomy but also when tympanoplasty is planned at a later stage.

However the pedicled grafts may often be too small and too short to fill the cavity completely For instance, the caudally pedicled sternocleido-mastoid muscle can hardly reach the antrum and the cranially pedicled temporal muscle can hardly fill the tip of the mastoid process. The object of the present study was to demonstrate the results of using a large, long flap composed of the retro-auricular subcutaneous tissue plus the temporal muscle.

MATERIAL AND METHODS

The material comprises 110 ears with active, chronic otitis. In 36 cases the suppuration had lasted 1–5 years, in 74 cases for more than five years. The perforation of the drum was central in two cases and peripheral in all the others. In 31 cases the defect was in the pars flaccida, in 79 cases in the pars tensa, including 29 very large or total perforations. At operation 83 patients were found to have cholesteatoma, 27 granulating processes in the antrum, mastoid process, or middle ear In 17 instances the ossicular chains were intact, in the others interrupted.

Our method of obliterating the cavity is a modification of Rambo's

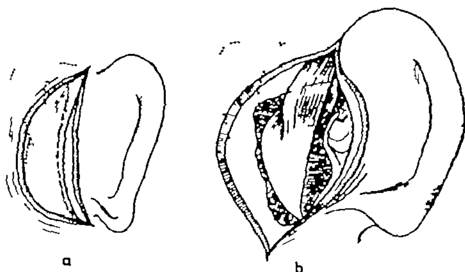


Fig 1 ( ) Incision of the *retro-auricular* tissue and temporal muscl ; (*b*) the flap of subcut neous tissue is placed int the ca ity so that one part fills the antrum nd the other part the tip of the mastoid process.

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RESULTS

Some 72 ears had dried 3–5 weeks after the operation, 16 ears 6–10 weeks, 9 ears 11–20 weeks, and 10 ears more than 20 weeks after the operation, while in three ears the suppuration never stopped. In 50 cases the cavity had been completely obliterated and presented only as a widened auditory canal. In 54 cases there was a small cavity while in six cases there was a large cavity indicating that the greater part of the flap had undergone necrosis.

In order to avoid retention of the cavity and to get a good view of the drum, we have laid great stress on widening the auditory canal. Three months after the operation the auditory canal and the external auditory meatus were widened in 86 cases. In 13 cases the auditory canal and the meatus were of the same size as prior to the operation. In eight cases the external meatus was rather narrow but yet the cavity and drum could be viewed. In two cases the external meatus was very narrow and this gave rise to marked retention of cerumen.

Postoperative complications occurred in five cases. Two patients developed, a few days after the operation, an abscess in the scar, one a haematoma at the donor site in the temporal region, and two had oedema of the temporal region. The abscesse were incised, the haematoma punctured, and the oedema subsided spontaneously. There have been no other complaints from the donor site, in particular no pain or disturbances of mastication.

To establish the results at long sight, and to ascertain whether cholesteatoma or infection beneath the subcutis-muscle flap would recur because of the obliteration of the cavity, the patients were recently seen in a follow-up examination. Out of the 110 operated ears only 103 could be examined. The average follow-up period was about 2 / years. In 59 cases it was one half to two and in 44 cases from 2–8 years.

The late results in the 103 cases showed that 82 ears had been dry ever since the operation, and the patients have not had any aural complaints. In 11 cases there had been intermittent aural discharge during the first 6–12 months after the operation, but thereafter it had ceased: the ears were found to be dry and the cavities were epithelialized at follow up. The average follow up period in these 11 cases was 31 months. In five cases there had been intermittent, and in one case constant, aural discharge, and at follow up there was suppuration in the ear. It came from the middle ear as the cavities were dry and epithelialized. Three patients reported that they had not had aural discharge but at follow up suppuration was present in the cavity while the drum was intact. Only in one case did discharge start more than one year after the operation after having been absent for a whole year.

The width of the auditory canal and the size of the cavity was the same

TABLE 1 *Postoperative air-conduction averages in frequencies 125–2000 cps.*

	Number of cases in various forms of tympanoplasty				
	I	II	III	IV	Total
Normal hearing (0–20 dB)	7	9	3	0	19
Good hearing (21–30 dB)	4	6	9	3	22
Hearing improved more than 15 dB	1	4	6	2	13
Hearing improved from 0 to 10 dB	4	3	5	3	15
Unchanged hearing	0	1	8	1	10
Hearing made worse from 0 to 10 dB	0	0	3	0	3
Hearing made worse more than 15 dB	1	0	2	0	3
Total	17	23	36	9	85

temporal musculoplasty The flap of the temporal muscle is usually too short to reach the floor of the antrum or the tip of the mastoid process. The use of the retro-auricular tissue combined with the temporal muscle gives a long cranially pedicled flap having two parts. One part is placed into the antrum, the others towards the tip of the mastoid process (Fig 1)

In the great majority of cases we used retro-auricular incision in a few cases Lempert s or Heermann s The retro-auricular incision was applied about $1^1/_2$ cm behind the attachment of the auricle. The skin was detached from the subcutaneous tissue which was then incised further anteriorly (Fig 1 *a*) so that later the subcutis was applicable as a pedicled graft 85 patients had modified radical operation with tympanoplasty and closure of the perforated drum with fascia Thirteen had modified radical operation closing the tympanic defect but without reconstruction of the defective ossicular chain The remaining 12 cases had the conventional radical operation To avoid later narrowing of the auditory canal the mastoidectomy and tympanoplasty were followed by dilatation of the auditory canal which was incised at 10 o clock through its entire length The incision was carried right through the external auditory meatus which was thus also dilated The auditory canal was packed with gelfoam and hydrocortisate terramycin meche

The named flap of subcutaneous tissue plus muscle was obtained by an incision as shown in Fig 1 and, together with periosteum, detached from the skull With scissors it was then cut so as to make two parts on one pedicle One part was placed into the antrum and the other part into the mastoid process (Fig 1 *b*) The fascia covering the tympanic defect also covered the bottom part of the muscle flap

Postoperatively antibiotics were administered, as a rule penicillin The meche was changed for the first time on the 10th day thereafter every 3rd day until the ear was dry

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MORPHOLOGIC CHANGES IN VESTIBULAR HAIR CELLS IN A STRAIN OF THE WALTZING GUINEA PIG

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Genetically deaf animals have been known for many years. Some of these animals combine deafness with a waltzing behaviour. In the present work some characteristics of the morphology of the vestibular hair cells from labyrinths of waltzing guinea pigs of various ages were studied with electron microscopical methods. In survey studies with low magnification clear pathological changes were found in labyrinths from adult animals: severely degenerated sensory cells and cellular debris pushed out into the endolymphatic space. With high resolution even the sensory cells of the newborn animals were found to be pathologically changed with variation in sensory hair diameter and balloonshaped protrusions of the sensory cell apical cytoplasm. In the sensory cells of type I a specific rod shaped cytoplasmic inclusion was regularly present. This inclusion body was composed of an electron dense substance seemingly consisting of fine tubules or fibrils. The synaptic region was normal in the sensory cells as long as the degeneration of the cell was not very advanced. Both afferent and efferent nerve endings were normally present. The morphological changes demonstrable in the vestibular sensory cells already from the birth lead us to believe the waltzing behaviour to be closely related to this peripheral sensory cell damage. The failure of earlier investigators to demonstrate pathological changes in the sensory epithelia is considered due to the limitation of the light microscopical methods used.

The occurrence of genetic deafness in animals has been recognized for more than 60 years, but the study of such animals has become increasingly rewarding in the last few years through the recent investigations on hereditary deafness in man and the modern morphologic and physiologic methods applied in inner ear research.

In some of these animals the deafness is accompanied by waltzing behaviour. As early as 1929 Ibsen reported on a strain of the waltzing guinea pig, and early morphologic studies of the inner ear of these animals were carried out by Lurie (1939, 1940), Lurie and Dempsey (1939), Cogan (1940).

Four guinea pigs donated to this department by National Institutes of

This work was supported by U.S. Public Health Service grant No. NB 03312-05-06, the Swedish Medical Research Council, and Harald and Greta Jeanssons Stiftelse.

at follow up as at conclusion of the treatment In particular there had not occurred further narrowing of the external meatus

As mentioned above, type I–IV tympanoplasty was performed in the same stage as mastoidectomy The average hearing of the frequencies 125–2000 cps two–three months after the operation is shown in Fig 2 A total of 41 patients (48.2%) obtained good or normal hearing (30 dB or more) Out of the remaining 44 patients, who did not gain more than 30 dB postoperatively 13 had gained more than 15 dB and 15 0–10 dB

ZUSAMMENFASSUNG

Somit ist eine Modifikation der Temporalis-Muskelplastik von Rambo zur Verödung der Mastoidhöhle beschrieben und die sofortige sowie spätere Operationsergebnisse bei 110 aktiven chronischen Mittelohrentzündungen dargestellt. Bei Anwendung der retroaurikulären Gewebe zusammen mit dem Musculus temporalis bekommt man einen langen kranial gestielten Lappen von welchem man einen Teil in den Antrum und den anderen zur Spitze des Processus Mastoideus einführt Um die Stenose des Gehörganges zu vermeiden, schneidet man die Haut des Gehörganges und des Meatus akustikus ekternus in der ganzen Länge auf

Bei dieser Methode wurden die Ohren bei über 90% der Fälle trocken und wir hatten wenige postoperative Komplikationen. Spätresultate zeigten, dass die Ohren trocken blieben und dass es nicht zum Rezidiv des Cholesteatomes oder zu Rezidiventzündungen kam. Die Ergebnisse der Gehörverbesserung werden kurzfassend dargestellt.

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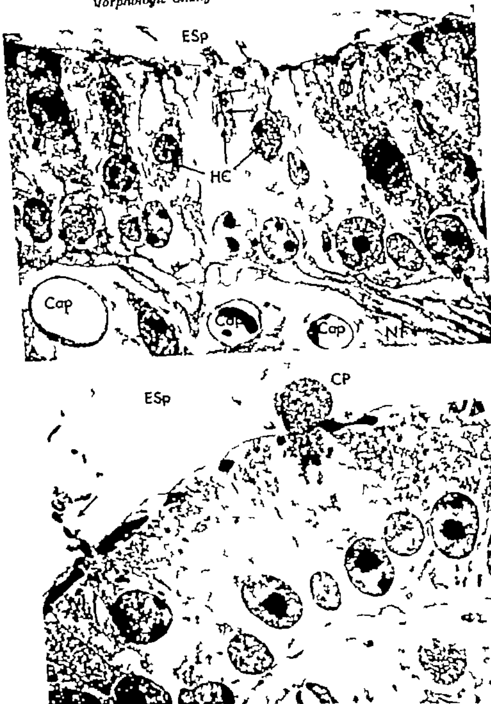


Fig. 1 Electron microscopic survey of crist mpullaris from 2-day-old (A) and an 11-months-old (B) w ltal g guinea pig. Not the decrease i the mber of the hai cells (A Protrusions of the hai cell t pe ce be f und here. A nerv fibre (NF) i see penetrating the basement membrane nd loosing it myelin sheath. In one of the hai cells of type I inclusio red is i ible (arrows) Capillaries (Cap) are found i the connecti tissue ESp, Endol mphatic space. HC, Hai cells. (B) Only few hair cells are still present. The hai cell top i found i protrude into the endolymph with balloon-shaped cytoplasmic projections (CP) nd the base of the sensory ha rs re clumped together (arrow) (A) 1875 1500, (B) 775 2700.

Health in 1961 have since been bred with normal "Swedish" guinea pigs, this providing the opportunity to analyse the genetic characteristics, physiology and morphology of these animals. The present study was undertaken to examine some characteristics of the morphology of the vestibular hair cells from the labyrinths of waltzing guinea pigs of various ages.

METHODS

Light Microscopy

The waltzing guinea pigs ranged in age from newborn to 2 years old. Fixation was performed as follows After inducing anaesthesia with Nembutal the thorax was opened a cannula was inserted in the ascending aorta, and perfusion was carried out with isotonic saline followed by the Heidenhain-Susa fixative solution After perfusion with approximately 500 ml the animal was decapitated, the head immersed in fixative and subsequently embedded by the celloidin technique (Lundquist, 1965) The specimens were orientated with the modiolus of the cochlea in the same plane as, and parallel to the knife 15 μ serial sections were cut on a celloidin microtome These were stained with haematoxylin and eosin and by the Mallory azan method

For detailed morphologic examination 1 μ sections were cut from Epon embedded specimens, stained with toluidine blue and examined by light and phase-contrast microscopy

Electron Microscopy

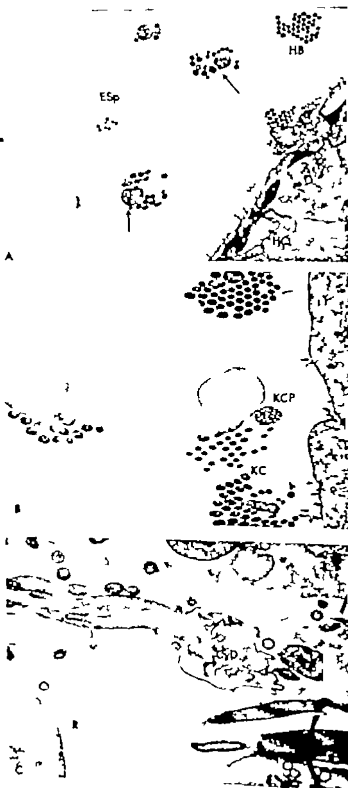
After anaesthesizing with chloroform and decapitation the temporal bones were dissected and the bullae tympanicae opened The round window was extirpated, the stapes removed, and the apex of the cochlea opened The specimens were then perfused with 1 per cent osmium tetroxide solution (Rhodin 1954) dissected under an operating microscope, and the various parts of the labyrinths embedded by the standard Epon procedure (cf Luft 1961 Kay 1965 Sjöstrand 1967)

After sectioning on a LKB Ultrotome the specimens were stained with uranyl acetate and lead acetate (Watson, 1958 Karnovsky 1961) and examined with a Siemens Elmiskope I For photography Gevaert Scientia 23 D 50 plates were used with primary magnifications ranging from 1000 to 40 000

RESULTS

Light Microscopy

The celloidin sections showed no significant changes of the morphology of the semicircular canals, ampullae utricle, and saccule as seen in the light microscope The ampullae were provided with a normal cupula. The sensory epithelia in the older animals, however showed a suspected reduc


HB
ESp
A
KCP
KC
B
SD

tion in the number of sensory cells but the celloidin sections did not allow a satisfactory analysis of the relation between the number of sensory cells and supporting cells.

The same was true for the epithelium on the saccular and utricular maculae

Electron Microscopy

Epithelial cytoarchitecture

A general examination of the vestibular sensory epithelia at low magnification disclosed no conspicuous malformations in the newborn animals. There seemed to be a normal relationship between the types I and II sensory cells (Wersäll 1956) In the older animals, however even the survey pictures showed the presence of definite pathologic changes, with a considerable decrease in the number of both types of sensory cells. Severely degenerated sensory cells and cellular debris were found between the supporting cells and in the endolymphatic space on the surface of the epithelium. A comparison between newborn and older animals indicated an obviously progressive degeneration of the sensory cells. The supporting structures were apparently normal and nerve fibers were identified (Fig 1)

Sensory hairs

Even in the newborn animals a slight variation in the hair diameter was found (Fig 2 A) The diameter of the kinocilia was normal Examination of their tubular filaments disclosed the absence of central tubules in some cilia and supernumerary tubules in others Balloon shaped protrusions of the apical cytoplasm were found in the majority of the sensory cells, usually in the periphery of the bundle

A pronounced distortion of the hair bundle configuration which increased with age was observed (Fig 2 *A–C*) In many of the animals the normal morphologic polarization of the sensory cells (Wersäll *et al* 1964) was severely disturbed The sensory hairs varied widely in appearance, sometimes with grotesque deformities In the greatly swollen hairs the plasma membrane was often ruptured. Hair remnants were found in various stages of disintegration

Hair cell top

In the newborn animal the density and configuration of the cuticle of the sensory hair cells was apparently normal The hair rootlets were inserted

Fig. 2. This montage illustrates the progressive degeneration of the sensory hairs (*A*) Newborn with varying diameters of the hairs (arrows) Sometimes a normal appearing hair bundle is found (*HB*) (*B*) With increasing age (5 days) increasing disconfiguration is found, and the kinocilia (*KC*) have sometimes pathological appearance with supernumerary tubuli (*KCP*) (*C*) In advanced stage of degeneration (38 days) a rich profusion of cytoplasmic debris (*CpD*) is found. (*A*) 376× 4500, (*B*) 330× 14,300, (*C*) 127× 18,000.



Fig. 5. In cross cut section the rod-shaped structure is rounded in appearance and can be found close to the nuclei (arrows). The nuclear border at this region is indentated. The inclusion described is found only in the type I cell (*HC I*). NCh, Nerve chalice. SC, secretary cell. Age: 12 months. 2481 × 14,400.



Fi 4 Low pow r electron micrograph showing ha cell of type I (*HC I*) with rod-shaped in lu n body passi g cl se to and ind nting th nucl us (*ar ws*) Othe hair c ll of type I a w ll a upporting cells with th ir basally located nucleae (*SCNa*) are present Th aff rent n rve fibe f rming the n rv halice i seen penetrati g thro gh th basem nt m mbra (*M H*) Age 80 d y 142X 6000



Fig 5. I cross cut section the rod-shaped structure is rounded I ppearance and can be found lose t the nuclei (arrows) The nclea border at this region is ind ntated. The inclusion described I found ly in the type I cell (*HC I*) VCh, Nerv halice SC, secretory cell Age 12 months. 248V 14,600.



Fig. 6. (*A*) Th ucl p rtion f a se sory cell t p I (*HC I*) where the n l s (N) is asymmetri w th t a ght nuclea bord du to th hypass of rod haped stru ture (*R*) close t th cl u NCh Nerv chalice Sc secretory cell (*B*) The rod haped structure (*R*) is f n d i penet t deeply int th v pile regi n push ng bag" f hair c ll cytoplasm ahead NE Nerv ndings. Ag : 80 days. (*A*) 142N 13,600 (*B*) 142N × 21 600.



Fig. 7. Hair cell type I, subnuclear region. At the top of the picture the nucleus (N). Among the normally present cytoplasmic organelles such as mitochondria (*MI*) lysosome-like structure (*Ly*) Golgi apparatus (*GA*) and multivesicular body (MB) fibrillar condensation is found in the cytoplasm (*arrows*) similar in structure to that shown by the rod-shaped inclusion bodies. *NCh*, Nerve chalice. Secretory cell (*SC*) filled with granules (*G*). Age 58 days. 127V ×23,400.

Synaptic region

So long as the degeneration of the cell was not greatly advanced the synaptic region in the sensory cell was normal. Normal synaptic bars and vesicles were regularly observed (Wersäll *et al.*, 1967). Synaptic bars were found in the immediate neighbourhood of the rod-shaped inclusions in a few cells.

Nerve Fibers and Nerve Endings

The efferent and afferent nerve endings in the epithelium of the newborn and the older waltzing guinea pigs were apparently normal in number. There appeared to be no degeneration of either the nerve fibers or nerve



FIG. 8 D ta l of pical part of hair cell, type I A protrusi n i to th d lymphatic pace is s n t l g the t p of a rod shaped l el lo body (R) w ll normally occ rring rg ll s, uch as mitoch dri (MI) centriol (Cl) nd l sosom lik structure (Lg Ag 8 mo th 337\ 36,200

endings, and apparently normal endings were observed even in the areas where all sensory cells were completely destroyed and/or expelled from the sensory epithelium

Supporting Cells

The supporting cells contained a normal amount of secretory granules, mitochondria and Golgi apparatus. There were apparently no changes during or after the degeneration of the sensory cells.

DISCUSSION

Light microscopic studies of the labyrinth in the waltzing guinea pig revealed normal vestibular epithelia (Lurie, 1939). This, together with the absence of any vestibular responses to clinical tests have led several investigators to ascribe the waltzing behaviour to a centrally located lesion (Cogan, 1940). Experimental surgery in the mesencephalon has resulted in a waltzing-like behaviour (Lurie & Dempsey 1939).

The marked degeneration of the vestibular sensory cells with morphologically normal nerve endings observed in the present study would indicate, however, that the waltzing behaviour is closely related to this peripheral damage. The fact that the behaviour was found already from birth is consistent with the observation of the progressive peripherally located degenerative changes in the newborn animals. It seems probable that this degeneration starts prenatally. It is still an open question whether the neuroepithelia of the vestibular organ in these animals mature completely before onset of the degeneration.

The intracellular rod shaped inclusions have not been reported hitherto. Their substructure is suggestive of a filamentous or tubular arrangement. This might indicate that the rod is composed of a protein conceivably originating from a defect in protein metabolism. It is interesting to note that the formation of a presumably pathologic protein that is visible in the electron microscope can be initiated in cochlear hair cells by kanamycin intoxication as shown by Wersäll *et al.* (1967).

ZUSAMMENFASSUNG

Genetisch bedingte Taubheit bei Tieren ist seit vielen Jahren bekannt. Einige dieser Tiere haben neben ihrer Taubheit auch eine eigenartige, walzend-ähnliche Art zu laufen. In dieser Arbeit wurden einige morphologische Besonderheiten der Vestibularis-Haar-Zellen im Labyrinth walzender Meerschweinchen verschiedenen Alters mit elektronenmikroskopischen Methoden untersucht. Bei Übersichtsstudien mit geringer Vergrößerung fand man deutliche pathologische Veränderungen im Labyrinth von erwachsenen Tieren, z.B. stark degeneriert Sinneszellen und Zelldetritus, der in das Spatium endolymphaticum ausgestoßen wurde. Mit starker Vergrößerung fand man sogar die Sinneszellen neugeborener Tiere pathologisch verändert mit Abweichungen im Durchmesser der Sinneshaare und ballonartigen Ausstülpungen des apikalen Zellplasmas. In den Sinneszellen des Typ I fand man regelmäßig einen spezifischen stabförmigen Zelleinschluß. Dieses Einschlußkörperchen schien aus einer elektronendichten Substanz zu bestehen, dem Anschein nach feinen Tubuli oder Fibrillen. Der Synapsbereich der Sinneszellen war normal, solange die Zelldegeneration nicht allzu fortgeschritten war. Sowohl afferent als auch efferent Nervendigungen waren normal. Die schon von der

Geburt erkennbaren morphologischen Veränderungen der Vestibularis-Sinneszellen machen es wahrscheinlich daß die abweichende Art zu laufen auf einen periferen Schaden der Sinneszellen beruht. Daß man früher keine Veränderungen in den Vestibularis-Sinneszellen walzender Meerschweinchen fand beruht auf dem begrenzten Auflösungsvermögen des Lichtmikroskopes.

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HORIZONTAL NYSTAGMUS OF RHESUS MONKEYS

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Horizontal optokinetic nystagmus (OKN), caloric nystagmus, and positional alcohol nystagmus (PAN) of the rhesus monkey (*Macaca mulatta*) were surveyed using electrooculography. OKN of the monkey appears to be similar in most respects to that of man although optokinetic after-nystagmus is more prominent in the monkey. Of the parameters of OKN which were measured, slow phase velocity was most closely related to the optokinetic stimulus. Caloric nystagmus is easily induced in the monkey and parameters of caloric nystagmus declined exponentially on repeated testing. The first phase of positional alcohol nystagmus was present in all animals tested and the secondary phase in some. The data indicate that oculomotor findings in the monkey can probably be applied to man with few reservations.

The monkey is a good subject for oculomotor studies. Both caloric and optokinetic nystagmus can easily be elicited in the monkey (Krieger & Bender 1956, Shanzer & Bender 1959) and its nervous system is anatomically similar to that of man (Ariëns Kappers *et al.* 1960, Snider & Lee, 1961). Recent papers have analyzed saccadic and pursuit movements of the stump-tailed monkey, *Macaca speciosum* (Fuchs, 1967), and the threshold for rotatory nystagmus of the rhesus monkey, *Macaca mulatta* (Lange, 1967). This paper is a preliminary survey of various types of horizontal nystagmus of the normal rhesus monkey with particular emphasis on optokinetic nystagmus. The purpose is to provide a basis for comparing nystagmus of monkeys before and after lesions of the central nervous system and aid in comparisons of the oculomotor system of monkey and man.

METHODS

Juvenile monkeys of the species, *Macaca mulatta*, weighing 2.5–3 kg served as experimental subjects. During testing, monkeys sat in a primate chair with arms and legs restrained and head held by a sponge-covered clamp. Animals were examined in light for saccadic movements, spontaneous nystagmus, gaze nystagmus, and optokinetic nystagmus (OKN). They were tested in darkness for spontaneous nystagmus, positional alco-

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hol nystagmus, caloric nystagmus, and optokinetic after nystagmus (OKAN)

The *optokinetic stimulus* was an internally lighted rotating drum, 36 inches in diameter with 1 inch black stripes at each 45 (Krieger & Bender 1956) The drum filled the monkey's field of vision and rotated at a *constant angular velocity Drum velocity was servo-controlled to maintain* a constant rate of rotation The rate of rotation was calibrated by counting the stripes which passed a photocell Eight stripes represented 360 of drum *rotation in the normal testing situation In two experiments (Fig 4 C D)* the number of stripes was doubled Caloric nystagmus was induced by injecting 15 cc of water at 27.5 C and 47.5 C into the external auditory canal *for 15 seconds This is 10 degrees above and below the monkey's body* temperature The head was extended so that the horizontal semicircular canals were vertical Caloric nystagmus and OKAN were recorded in darkness.

The recording system is demonstrated in Fig 1 The upper line is the time base at 1 mark/sec. The second trace is a photocell recording of the passage of black stripes The third trace is a recording of the corneoretinal potential (EOG) with DC-coupling through platinum needle electrodes inserted at the lateral canthi. The EOG amplifiers were chopper stabilized and had a bandpass from DC to 150 cps For recordings of Figs 1 6 and 7 filtering was added to attenuate frequencies above 22 cps. The recordings of Figs 2 and 3 and those used in determining the parameters of optokinetic nystagmus (Figs 4 and 5) were taken without these filters.

The fourth trace of Fig 1 is the differentiated EOG showing momentary angular eye velocity (Henriksson, 1955 1956) This trace was used to measure the velocity of quick phases of nystagmus. The differentiators had a high frequency *bandpass of 150 cps and a time constant of 3 msec. They* accurately phase shifted a 1 millivolt 20 cps sine wave introduced through the EOG input by 90 The maximum velocity of this signal was equal to that *of an eye movement of about 1800 /sec.* In the recordings of Figs 1 6 and 7 filters were added to the differentiators which reduced the maximum velocity of the eye movement signals passed without distortion to about 500 /sec.

The differentiated EOG was also amplified and rectified (fifth trace Slow phase eye velocity) to determine angular velocity of the slow phases of nystagmus in isolation By integrating the rectified slow phase eye velocity (sixth trace) total eye deviation was obtained When the integrator pen reached the limit of its excursion it returned to the baseline and started again This accounts for the "sawtooth nature of the integrated trace during nystagmus.

Nystagmus frequency is shown in the seventh trace in a recording of beat to beat frequency Considerable variability was usually present in beat to beat frequency so that beats of nystagmus were also counted separately for set periods of time The vertical bar on the right of the EOG trace

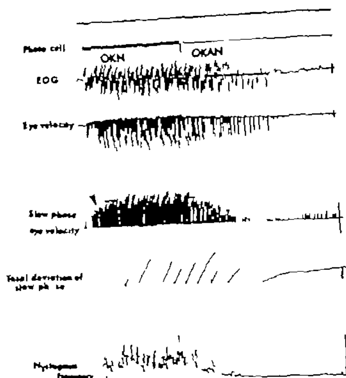


FIG. 1. OKN and OKAN of a normal monkey in response to an optokinetic drum rotating at 90°/sec. The time base is shown below at 1 mark/sec. The passage of each stripe caused a deflection in the photocell trace. The sharp downward deflection in the photocell trace shows the time when the lights were extinguished. OKN was recorded in darkness. The initial OKAN was to the left and was followed by small beats of OKAN to the right. The downward arrow over the initial portions of the slow phase velocity trace indicates the period during which the velocity of slow phases gradually increased up to about 90°/sec.

represents about 10° of eye deviation. The EOG was calibrated by alternately showing the monkey lights separated by 15° or 30° in a dimly lit room and averaging a large number of responses. Calibrations are approximate rather than absolute values. Vertical bars on the right of the differentiated traces are approximately 100°/sec and on the right of the integrated trace approximately 100° of deviation. In each of the recordings eye movements to the right caused upward pen deflections.

Parameters of OKN were measured only in animals which were consistently attentive to the optokinetic stimulus when alert. These were about 20 to 40% of the animals tested. A constant state of alertness was maintained by injecting 0.5 mg/kg of amphetamine sulfate intramuscularly at least 30 minutes before testing. Amphetamine does not change the characteristics of nystagmus (Collins & Poe 1962, Cohen *et al.* 1960). Two types of optokinetic stimulation were used in this study to induce nystagmus

1 Drum rotation at a constant velocity of 90 /sec for a period of 45 seconds as shown in Fig 1 At the conclusion of this period the lights were extinguished and optokinetic after nystagmus (OKAN) was recorded in darkness

2 Drum rotation for periods of 30–45 seconds at constant velocities between 30 –180 /sec. Step increases in drum velocity always progressed from lower to higher velocities for reasons which will be given subsequently

RESULTS

The response of a normal monkey to a 45 sec period of constant optokinetic drum rotation at 90 /sec (2 stripes/sec) is shown in Fig 1 The first eye movement was a slow phase to the right followed by quick phases to the left The eyes beat across the midposition during most of the OKN The approximate midposition is shown by the dotted line. The amplitude of the beats varied between about 10 and 30 Quick phase velocities varied but generally did not exceed 400 /sec.

In this as in other animals the eyes did not attain the greatest slow phase eye velocity immediately after the stimulus began. Instead over the first 10 seconds of OKN the maximum velocity of the individual slow phases gradually increased This period is marked in Fig 1 by the downward arrow over the slow phase eye velocity trace The frequency during this time was relatively constant Because of the initial lag in attaining high velocity slow phases, the response to optokinetic stimuli was measured after the first 15 seconds of OKN and the speed of drum rotation was increased from lower to higher velocities.

After the first 10 seconds of OKN the maximum velocity of slow phases varied around 90 /sec. The dotted line through the top of the Slow phase eye velocity trace shows approximately 90 /sec. Integration of slow phase velocity indicates that the eyes moved about 2250 during the 45 sec period of optokinetic stimulation while the drum was travelling 4050 If the initial 15 seconds of OKN is disregarded, the eyes travelled about 1650 during slow phases in the last 30 seconds of OKN while the drum was travelling 2700 The mean duration of quick phases was about 50 msec (unpublished data) and the mean frequency was about 4 beats/sec If 20% is subtracted from 2700 for time spent in quick phases, then 2160 is the arc traversed by the rotating drum during the time the eyes were moving 1650 in the slow phases.

The basis for the difference in the angle of drum rotation and of eye deviation during slow phases of OKN was probably largely due to the fact that the eyes did not move with constant velocity during the slow phases. Occasionally slow phases were of constant velocity particularly at lower OKN drum velocities More often, however the velocity of slow phases was not constant but fell steadily as the beats progressed (Fig 2, 53 /sec) At higher drum speeds (98 /sec) the velocity of slow phases fell steadily in

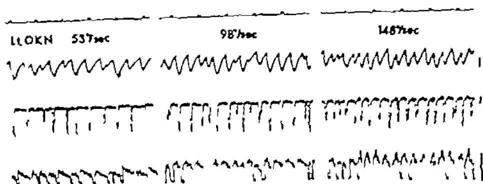


Fig. 2. OKN of normal monkey. The top trace is the time base 1/sec. The second trace is the EOG, the third trace the differentiated EOG, and the fourth trace the differentiated EOG with the velocity of the quick phases clipped to show slow phase velocity in isolation. The velocity of the OKN drum is marked above each series of traces. Calibrations as in Fig. 1.

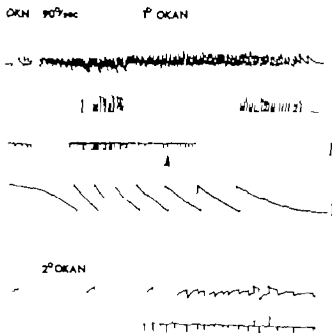


Fig. 3. OKN and OKAN of a normal monkey. The lights were extinguished at the downward arrow. The recording scheme is similar to that in Fig. 1 except that only the EOG and differentiated EOG are shown during secondary OKAN (2° OKAN). The recordings are continuous. Note the relatively linear decline in slow phase velocity during primary OKAN (upward arrow).

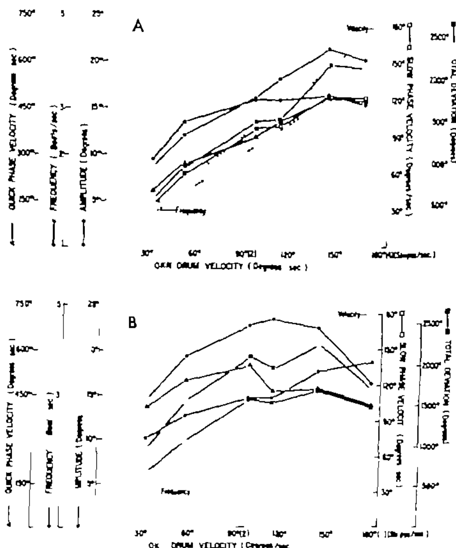
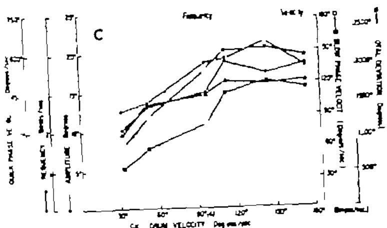


FIG. 4 Graphs f par m t rs of OKN of ne m nkey Craph *A* and *C* were obtained from data recorded on the same date and *B* and *D* f om dat taken 5 days lat r *A* nd *B* are the tandard test itu tion with eight stripes on th OKN drum. In *C* and *D* the number of tripes wa doubled. Each point i th m a alue of a single parameter er a 30-sec nd period The locity and frequ ney of th OKN timulus are shown on the abscissa in degrees/sec nd tripes/sec. Perfect following fo sl w phase locity in degre s/sec o fo frequ ncy in beats/sec w uld f llow th dott d lines marked "Velo c ty and Frequ ncy" The closed boxe are symbol fo total deviati n in degrees, th pen boxes f ma imum l w phase velocity n degrees sec, th open triangles f r max mum quick pha e locity in degrees sec the losed circle f mplitude f beats in d grees, nd th pen circles f frequ ncy in be t sec

some beats while in other it reached a maximum somewhere in the middle of the beat At still higher drum speeds (148 /sec) eye velocity initially rose and then fell in most beats. With prolonged stimulation at higher drum velocities the velocity configurations often varied, there being a mixture of beats with rising and falling velocities

Beats of OKN with slow phase velocity which appeared to fall exponentially have been described in the cat (Honrubia *et al.*, 1967) although spe-





cific tests for exponentiality were not performed. Beats with similar configuration also occurred during slow nystagmus in the monkey e.g. during the OKAN of Fig. 1 and the secondary OKAN of Fig. 3. During more frequent nystagmus these beats were much less common than other varieties of slow phases which have been described.

Optokinetic After Nystagmus (OKAN)

Optokinetic after-nystagmus (OKAN) with quick phases in the same direction as the preceding OKN is prominent in the monkey in darkness (Krieger & Bender 1956 and Figs. 1 and 3). In light OKAN is of short duration or is absent. OKAN was found in every alert monkey which had OKN. OKAN generally lasted for about 60–90 seconds after the end of OKN (Krieger & Bender 1956). The period of OKN necessary to elicit full-duration OKAN varied in different animals. In some monkeys 10–15 seconds of OKN were sufficient to induce OKAN of full duration. Periods of OKN longer than 30 seconds did not lengthen the period of OKAN. Postrotatory nystagmus is not similarly prolonged in darkness in the monkey.

During OKAN the amplitude of the beats was relatively constant but both the frequency and maximum slow phase velocity slowly declined. Usually but not invariably the decline in maximum slow phase velocity during OKAN was linear (Fig 3 upward arrow slow phase velocity trace) The maximum slow phase velocity of the beats at the onset of OKAN was usually somewhat lower than the velocity of the slow phases of OKN which had just preceded them (Fig 3) Primary OKAN i e. that with quick phases in the same direction as the preceding OKN was followed by secondary OKAN with quick phases in the opposite direction in some animals (Figs. 1 and 3) Secondary OKAN also occurs in humans (Morimoto *et al* 1963) In the animals whose records are shown in Figs. 1 and 3 oppositely directed primary and secondary OKAN were induced when the direction of the stimulus was reversed

Parameters of OKN

Since a randomly selected group of monkeys was not studied, no attempt was made to statistically define the normal range of OKN in the monkey Instead the response of several apparently normal monkeys with very good OKN was studied. The relationship of parameters of OKN of one of these monkeys to six different speeds of drum rotation between 30 and 180 /sec is graphed in Figs 4 and 5 Samples of OKN from this animal are shown in Fig 2

Despite some variability in each of the parameters of OKN recorded in the same animal on different days (Fig 4 A *B*) within limits as the velocity of the OKN drum increased, each parameter of nystagmus also tended to increase (Fig 5) The beats of nystagmus became more frequent, the average amplitude of the beats became larger the maximum quick and slow phase velocities increased and the eyes deviated farther in response to the stimulus.

In this as in other monkeys with active OKN the eyes were able to approximate the velocity of the optokinetic drum during the slow phases of OKN up to drum velocities between 90 and 120 per second In each of these animals the maximum velocity of some of the beats of OKN exceeded the velocity of the OKN drum at speeds up to 90 /sec In Fig 4 *B* this tendency was pronounced and the mean of the maximum velocities (open squares) was higher than velocity of drum rotation up to 90 /sec. This was also reflected in a higher total deviation of the eyes at drum velocities up to 90 /sec in this instance (Fig 4 *B* closed squares) OKN following at slow phase velocities up to 90 /sec has also been reported in man (Gruttner 1939 Ueda & Suzuki, 1965) Between 120 and 150 /sec the maximum velocity of the slow phases usually increased along with speed of drum rotation, but the eyes did not approximate the velocity of the OKN drum (Fig 5) Above 150 /sec the maximum velocity of the slow phases often fell. For this reason 180 /sec was used as the upper limit of drum velocity for testing

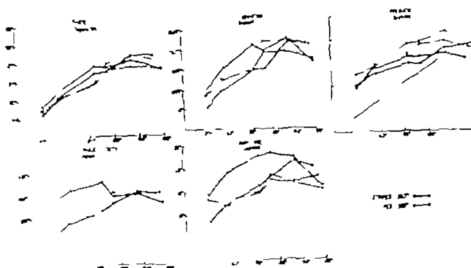


FIG. 5. Dat of Fig. 4 replotted for individual parameters. The solid lines in each graph show the response t the normal test situation (8 lines/360°). The dotted lines show the response to doubling the number of stripes in the OKN drum. The underlying dotted line in the graph of slow phase v elocity shows perfect following for ach elocity of drum rotation. In the graph of freq ency the underlying solid line show frequency changes which would occur if one beat of ystagmus corresponded t the passag of ne stripe whe there w re 8 stripes/360 The dotted line shows 1 1 following for 16 stripes/360°

Changes in total deviation (Fig 4 closed squares, and Fig. 5) paralleled hanges in maximum slow phase velocity That is, the curves tended to be linearly related to the stimulus up to about 90 to 120 /sec. Between 120 and 150 /sec the curves generally increased but not at the some rate as the stimulus. Above 150 /sec total deviation fell There was more variation in total deviation than in maximum slow phase velocity Average amplitude of the beats of OKN (Fig. 4 closed circles, and Fig. 5) also increased with increases in drum velocity Curves for quick phase velocity (Fig. 4, open triangles, and Fig. 5) roughly paralleled those for average amplitude at drum locities up to 90 /sec. This might be expected since there tends to be a linear relationship between the two at lower amplitudes in monkeys as in humans (Dodge & Cline, 1901 Goto et al., 1968 Cohen et al., 1969)

The frequency of OKN (Fig. 4 open circles, and Fig. 5) also rose with increases in drum velocity It varied between 1.5 and 4 beats/second. The average frequency of h rizontal OKN or caloric nystagmus generally did not xceed 3 beats/sec in the normal monkey whatever the stimulus. When testing with 8 stripes f r each 360 of rotation, the frequency of OKN (open circles, Fig 4 A B) was higher at lower drum velocities than the actual number of stripes which passed the eyes (dotted line, "Frequency") This uggests that the animal was responding more to the velocity of the drum than to the number of stripes which were passing the eyes. This is

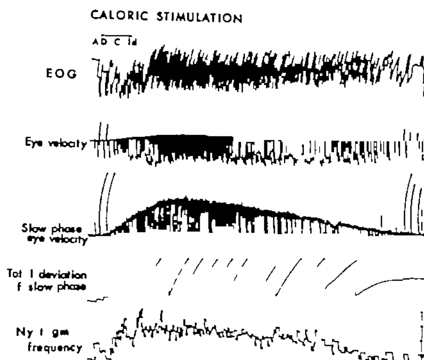


Fig 6 Cal ric nystagmus of a normal monk y induced by 10 seconds f irrigati n of the right ear with cold water (27.5 C) The period of stimulati n i hown by the horizontal bar between the first and second trac s. The lights w re extinguished after th end of cal ric irrigation and th rest of the record was taken in darkness. Calibrations as in Fig. 1 except that th vertical bar for the slow phase eye elocity trace represent 60 /sec.

in agreement with findings in other studies in animals and man (Gruttner 1939 Honrubia *et al* 1967)

When the number of stripes in the drum was doubled, maximum slow phase eye velocity did not substantially change (Fig 4 *C D*) Frequency of nystagmus increased slightly at higher speeds of drum rotation but not in direct proportion to the increase in the number of stripes The amplitude of beats tended to be slightly lower when the number of stripes was doubled There was no consistent change in total deviation

Caloric Nystagmus

As in humans (Jongkees & Philipszoon, 1964) 10–15 seconds of stimulation are sufficient to induce 2 to 3 minutes of brisk caloric nystagmus in the monkey In the example shown in Fig 6 the eyes initially beat to the left of the midline, i e on the quick phase side. 15 seconds later the beating field was across the midline The tendency of the eyes to beat on the quick phase side of the midline during OKN has been described in man by Gruttner (1930) and Mackensen & Uber (1960) Recently Hood (1967) noted that the eyes also beat primarily on the quick phase side during caloric nystagmus in man The significance of this phenomenon is not known This tendency was marked during the nystagmus of Fig 3 and was present briefly in the initial portions of the nystagmus of Figs. 1 and 5 For the

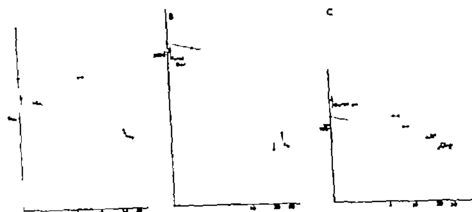


Fig. 7 Graphs showing the decline in various parameters of the oculomotor response of normal monkey to repeated caloric testing. The stimulus was 15 cc of water at 27.5°C delivered to the same ear over 15 seconds. Five minutes were allowed to elapse between trials. Trials are listed sequentially on the abscissa on a logarithmic scale. The scale of the ordinate is linear. The ordinate in A is maximum slow phase velocity at the peak of the caloric response. In B the ordinate is total deviation of the eyes in response to the stimulus, and in C duration of the caloric response.

most part, however during both the OKN and caloric nystagmus shown in Figs. 1 and 3 the eyes beat across the midposition.

At the peak of the caloric response (Fig. 6) the beats of nystagmus were about 30 in amplitude, the maximum slow phase velocity was about 105 / sec, and the average frequency was about 3 beats per sec. The amplitude and maximum quick phase velocity of this nystagmus were relatively constant for almost 60 sec during the middle of the response. Frequency had some tendency toward culmination (Torok, 1957) but was relatively constant at between 2 and 3 beats/sec for about 30 seconds during the middle of the response Maximum slow phase velocity continuously declined after reaching a peak about 30 seconds after the onset of stimulation. The eyes deviated about 6300 in response to the stimulus. Secondary phases of caloric after-nystagmus were commonly present.

In monkeys as in humans, cats and dogs (Crampton & Schwam, 1961 Collin & Updegraff 1966) there was a striking response decline to caloric stimulation. This is shown graphically in Fig. 7 Response decline usually began after several trials for each of the parameters of the nystagmus which were measured and appeared to fall exponentially Brown & Marshall (1967) have noted the exponential nature of response decline curves in cats. The slope was greatest for maximum slow phase velocity (Fig. 6 A) and total deviation of the eyes in response to the caloric stimulus (Fig. 6 B) If these curves were extrapolated, they would cross zero at about 110 trials. There was also a steady decline in the duration of the induced nystagmus (Fig. 6 C) but the slope of the response decline curve was shallower cross-

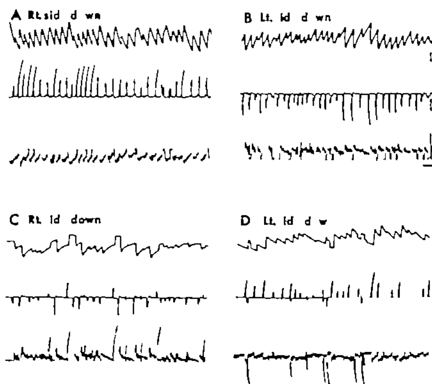


FIG. 8 P sitional alcoh l nystagmus (PAN) of a normal monk y The top trace in A D is the EOG recorded with RC-coupling (3-second time constant) the second trace is the differenti ted EOG and the third trace sh ws slow phase elocity Rectification was arti ficial in this case A nd B were record d 30 minutes after ingestion f alcohol and C and D were recorded 5 hours late The time base sh wn by the h riz ntal bar under B represents 2 seconds. Calibration in Fig. 1

ing zero at about 700 trials Differences in slopes of response decline curves for total deviation and duration of caloric nystagmus also appear to be present in data of Capps & Collins (1965) and Collins & Updegraff (1966) on cats and dogs.

Positional Alcohol Nystagmus (PAN)

Both primary and secondary positional alcohol nystagmus (PAN I and PAN II) were induced in the monkey PAN I was present in every animal tested, but PAN II was not constantly found As in humans (Aschan *et al.*, 1958 Bergstedt, 1961) the quick phases of PAN I were geotropic (Fig 8 A B) and of PAN II were apogeotropic (Fig 8 C D) In most animal PAN I developed about 20 minutes after ingestion of alcohol and persisted for about one and one half hours. When PAN II occured, it followed PAN I after a nystagmus free interval of from two to four hours. The nystagmus of PAN II reached a peak about 6 hours after ingestion and lasted for more than 4 hours PAN II has not been found in cats (Bergstedt 1961 Jong kees & Philipszoon, 1964) but has been reported in some white rabbits (Nohara, 1966)

DISCUSSION

Anatomically the structure of the oculomotor apparatus of monkey is close to that of man. Data in this report show that nystagmus of the rhesus monkey is similar in many respects to that of humans. In both monkey and man the best pursuit velocities are about 45°/sec (Robinson, 1965; Fuchs, 1967) while maximum following during slow phases of OKN is about 90°/sec (Gruttner, 1939; Ueda & Suzuki, 1965). Maximum quick phase velocities during caloric and optokinetic nystagmus are also comparable in monkey and man (Kolke, 1959; Mackensen & Schumacher, 1960) as are positional alcohol nystagmus and response decline on repeated caloric stimulation. It is likely that results of oculomotor testing in monkeys can be correlated rather closely with test findings in humans. Changes in eye movements after lesions of the oculomotor system also appear to be similar in monkey and man (Cohen *et al.*, 1968).

In both monkey and man the eyes are able to follow moving targets faster during OKN than during pursuit movements. Theoretically both pursuit movements and slow phases are induced by the same neural organizations (Rashbass, 1961). It is possible that a warm up period is necessary before the slow phase or pursuit system can move the eyes faster than 45°/sec. It is of interest that the eyes often move faster than the stimulus at some time during the slow phases and that the velocity of the slow phases is generally not constant. Perhaps the high initial velocity of slow phases is used to overtake the stimulus so that the eyes can approximate the velocity of the target more easily.

The major difference in the oculomotor system of monkey and man appears to be in OKAN. OKAN of long duration is sometimes found in humans with labyrinthine or oculomotor system lesions (Kirikae *et al.*, 1963). However, in the normal subject OKAN is brief (Gruttner, 1939) or cannot be elicited at all. In the monkey OKAN was found in every animal which had OKN and persisted for many seconds. *There was no sensory* input during OKAN and the response must have been entirely due to activity present within the central nervous system at the end of OKN. That is, neural organizations responsible for OKAN must have stored activity during OKN which was discharged in a regular fashion over the next minute or minute and a half. The neural substrate of OKAN is not known but probably involves the reticular formation of the pons and mesencephalon. Long-lasting defects in OKAN occur after lesions in these regions (Shanzer *et al.*, 1958; Cohen *et al.*, 1968).

A variety of parameters of OKN have been measured. Since slow phase velocity bears the closest functional relationship to the visual input, it should be the parameter of most value in input-output studies (Henriksson, 1956). Indeed maximum slow phase velocity showed the smallest variation of any of the parameters of OKN and was *directly related to the stimulus* over wider ranges than any other parameter. Only if maximum slow phase

velocity of individual beats of nystagmus is measured, can it be determined whether the eyes are able to approximate the velocity of a moving target.

Frequently however the maximum slow phase velocity of any beat was reached only momentarily Consequently a more general measure of slow velocity over a longer period of time would appear to be helpful in determining the quality of the response This is particularly true when the stimulus and/or the oculomotor response vary as during pendular rotation (Setoguchi & Suzuki 1965) the OKP test (Suzuki & Komatsuzaki, 1962) or caloric stimulation, or during spontaneous nystagmus. Because the velocity of individual slow phases varies a great deal, mean slow phase velocity of individual beats is not easy to determine Total deviation on the other hand gives some measure of mean slow phase velocity over time (Jung & Toennies, 1948) and can easily be determined electronically Total deviation also tended to be linearly related to drum velocity of up 90 /sec.

In contrast to maximum slow phase velocity and total deviation which appeared to be linearly related to the velocity of the optokinetic stimulus within limits other parameters such as amplitude, maximum quick phase velocity and frequency had a more complex relationship to the OKN stimulus. There was considerable variability in amplitude of beats of nystagmus and in its derivative, quick phase velocity Nevertheless these parameters are probably the most sensitive measure of the integrity of the rapid eye movement mechanism Limits of frequency are probably set by the characteristics of the nystagmus mechanism (Cohen *et al.*, 1965) and are related both to the number of stripes passing the eyes in the monkey and to background illumination (Valciukas & Pasik, 1968) Eye position probably also effects frequency and amplitude of OKN frequency being higher and amplitude lower when the eyes are beating on the quick phase side of the midline (Mackensen & Uber 1960) Changes in frequency amplitude and quick phase velocity of caloric nystagmus and OKN are characteristic after lesions of the pontine reticular formation (Cohen *et al.*, 1968)

Of the other parameters of nystagmus, duration of caloric nystagmus has probably been studied most extensively It is not much affected by central nervous system lesions (Henriksson, 1956 Jung & Kornhuber 1964 Cohen *et al* 1968) but has proven to be of value in diagnosis of peripheral labyrinthine lesions (Cawthorne *et al* 1956) Duration of OKN is arbitrarily controlled but duration of OKAN may be prolonged after peripheral labyrinthine lesions (Kirikae *et al.*, 1963) Since each parameter of nystagmus tends to increase as the stimulus becomes more intense any or all can give some measure of the intensity of the response (Koike, 1959 Torok & Derbyshire, 1968) However it is likely that each parameter has a slightly different contribution to make in diagnosis When the oculomotor changes produced by discrete lesions of the central and peripheral nervous system are quantitated and known, the diagnostic value of electrooculography should be considerably enhanced

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ZUSAMMENFASSUNG

Horizontaler optokinetischer Nystagmus (OKN), kalorischer Nystagmus und alkoholischer Stellungsnystagmus (PAN) des Rhesusaffen (Macaca mulatta) wurden mit einem Elektrookulographen untersucht. Der OKN des Affen erscheint im wesentlichen ähnlich dem des Menschen, obgleich der optokinetische Nach-Nystagmus mehr ausgeprägt beim Affen ist. Von den gemessenen Werten des OKN stand die Geschwindigkeit der langsamen Phase in engster Beziehung zur optokinetischen Reizung. Der kalorische Nystagmus ist beim Affen leicht induzierbar und bei dieser Gruppe war eine Abnahme des Nystagmus bei wiederholten Versuchen zu bemerken. Die erste Phase des alkoholischen Stellungsnystagmus war in allen untersuchten Tieren vorhanden und die sekundäre Phase in einigen der Versuchstieren. Die Angaben weisen darauf hin, dass die okulomotorischen Ergebnisse der Versuche beim Affen wahrscheinlich mit ein gem Vorbehalt beim Menschen angewandt werden können.

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RENAL ADENOCARCINOMA WITH METASTASES TO THE THYROID GLAND

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Four cases of renal adenocarcinoma with metastases to the thyroid gland are reported. In two cases the metastases developed almost simultaneously with the primary tumor and in the other two cases there was an interval of 12 and 16 years, respectively from nephrectomy to the appearence of metastases. Some of the peculiar features of renal adenocarcinoma are briefly discussed, as well as the incidence of metastases in general to the thyroid gland, their diagnosis and principles of treatment These four cases bring to 48 the total number of published cases of renal adenocarcinoma with metastases to the thyroid gland that we have found in the literature.

Renal adenocarcinoma[1] in humans is a neoplasia with some unusual features This tumor has lately been called "a clinical challenge" (Hewitt, 1967) and it has been stated that "malignant renal tumors should be classed with syphilis and tuberculosis as among the great mimics encountered in clinical medicine" (Creevy 1935) Some of the main reasons for the uniqueness of renal adenocarcinoma can be summarized in four groups

1 The symptoms of the primary tumor are various, often late and sometimes completely absent (Arner & von Schreeb, 1966 Böttiger *et al.*, 1966)

2 Any organ can be the site of secondary deposits This may be explained in part by the rich vascular supply to the kidney and the tumor (Mostofi 1967)

3 The time of development of metastases is even more unpredictable than in other malignancies, although there exists a fair correlation between the clinical signs, morphology of the tumor and staging of the disease on one hand and prognosis on the other (von Schreeb, 1967)

4 Both the primary tumor and the metastases have been claimed to show spontaneous regression (for discussion and reviews of the literature see Goodwin *et al* 1967 and Markewitz *et al* 1967)

The most common sites for secondary deposits from renal adenocarcinoma are Lungs, lymph nodes, skeleton and adrenals (Riches *et al.*,

[1] Synonyms Grawitz tumor hypernephroma, clear cell adenocarcinoma, granular cell carcinoma, to mention but a few

1931 Lucké & Schlumberger 1957 Mellcow & Uson, 1960) but as men tioned any organ can be involved and any clinical specialist may encounter a metastasis in "his" field.

Thus, the otolaryngologist may see secondaries from a renal adenocar cinoma in the paranasal sinuses (Hamberger 1943 Eneroth *et al* 1961) in the thyroid gland, or rarely in the mandible (Leskien, 1966) tongue, tonsils, or larynx.

In the thyroid gland, reported metastatic lesions from renal adenocar cinoma are rare. The first published case seems to be the one discovered at autopsy by Lubarsch in 1894 In 1954 Bruce & Michie published a sum mary of 22 previously reported cases and added two of their own To our knowledge, 20 further cases have since been reported (Illyés & Gerlei, 1954 (1 case) Hartmann, 1958 (1) Lange, 1960 (1) Mellcow & Uson, 1960 (2) Helmann, 1961 (2) Shimaoka *et al* 1962 (6) Wychulis *et al* 1964 (4) Freund, 1965 (1) Burge & Blalock, 1967 (1) Crocker 1967 (1)) Of these 44 cases, 16 have been detected at autopsy and 28 during the life-time of the patients.

In the Department of Otolaryngology and from the files of Radiumhem met, Karolinska Sjukhuset, we have collected four cases of renal adenocar cinoma with metastases to the thyroid gland

Case Reports

Case 1

The patient was an 88-year-old man who presented in January 1951 an acute onset of severe back pain on the right side in connection with vomiting and dark coloured urine Renal angiography demonstrated an orange-sized tumor in the lower part of the right kidney containing several pathological vessels. The sedimentation rate (SR) was 25 mm per hour

During this admission nephrectomy on the right side was performed. The low r part f the right kidney was occupied by an orange-sized tumor It was a circumscribed renal adenocarcinoma without any apparent infiltra tion of th renal pelvis or vessels. The carcinoma was moderately well differentiated and classified as malignancy grade II A according to Arner (Arner *et al* 1965) The tumor was radically extirpated

The patient remained asymptomatic for 16 years, but in the course of a check up at an eye clinic in F bruary 1967 a tumor unnoticed by the patient, was detected in th lower right part of the neck A needle biopsy from the tumor contained tumo cells with abnormal nuclei most compa tible with a metastasis from a renal carcinoma. I[131] tracing showed normal values, but the scintigram demonstrated a defect in the tumor area (Fig. 1) Radiographs of the chest and vertebral columna were normal. The patient was admitted to the Department of Otolaryngology in May 1967 in good general condition. He had a rather soft, non-tender mass, the size of a man s fist, in the lower right part of the neck (Fig. 2) Laboratory examinations

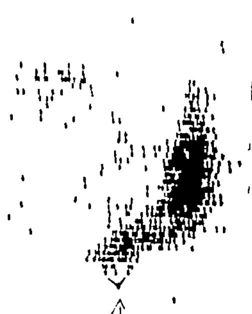


Fig. 1



Fig. 2

Fig. 1 Scintigram in ca e 1 an 88-year old mal perated 16 years previou ly f r a renal carcin ma on th right side A large defect i demonstrated in the l w part f the ight thyroid lobe

Fig. 2 The clinical finding in case 1 w a fist ized, rath soft, ind lent tumor corresponding to th lo r part of the right thyroid l be

showed hemoglobin (Hgb) 13 4 g/100 ml SR 22 mm per hour white blood cell count 3400 serum creatinin 1 0 mg/100 ml Three days after admission the thyroid gland was exposed via a collar incision, the right recurrent nerve was isolated and a hemithyroidectomy including the tumor was performed on the right side

The histopathological finding was a radically extirpated metastasis from a renal carcinoma to the thyroid It was classed as malignancy grade II A same as the primary tumor

At the last physical examination in August 1967 there were no signs of recurrence of the tumor or further metastases.

Case 2

The patient was a 40-year-old man In December 1966 he noted pain in the groin Urography in January 1967 showed an expansive process in the right kidney and renal angiography demonstrated a densely vascularized tumor (Fig 3) No metastases could be detected, and the left kidney was roentgenographically normal The SR was 66 mm/hour and Hgb 10 3 g/100 ml A needle biopsy from the tumor was attempted, but no tumor cells were obtained

Nephrectomy on the right side was performed in March 1967 The upper pole of the kidney was occupied by a grapefruit sized tumor which was

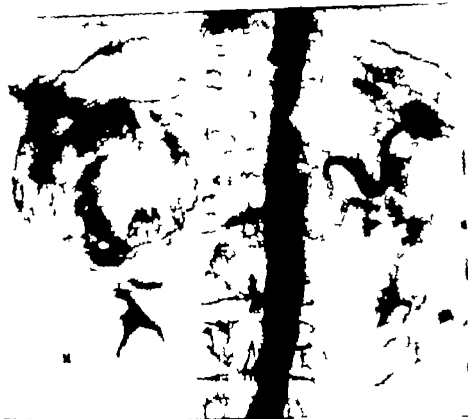


Fig. 2. Renal angiography in case 2. During the vascular phase several tortuous, pathological vessels were demonstrated intermingled with areas of very poor vascular supply.

ually removed. On the abdominal surface of the diaphragm a hazelnut sized tumor was detected and removed.

The tumor was a renal adenocarcinoma with the maximum diameter of 11 cm. The tumor removed from the diaphragm was a metastatically involved lymph node. Both were classified as malignancy-grade II B (Fig. 4). No invasion of the vessels of the kidney could be detected and the carcinoma as well as the metastasis had apparently been removed in toto.

In April 1967 the patient was admitted to Radiumhemmet for postoperative irradiation. On this occasion a plum-sized tumor was observed in the lower part of the right thyroid lobe. The patient stated that he had noticed the swelling right after the renal angiography 3 months previously. A needle biopsy from the tumor contained dense cell-complexes of rather monomorphous cells with pronounced vacuolization in the cytoplasm. The cytological interpretation was that the tumor probably was a thyroid adenoma with regressive changes, but a metastasis from a renal adenocarcinoma could not be excluded.

I[131] tracing showed normal values, but on the scintigram the lower part of the right lobe had a decreased uptake

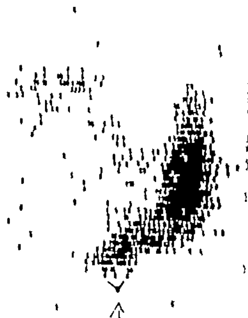


FIG 1



FIG. 2

FIG. 1 Scintigram in case 1 an 68-year old male operated 16 years previously for a renal carcinoma on the right side A large defect is demonstrated in the lower part of the right thyroid lobe

FIG. 2 The clinical finding in case 1 was a fist-sized, rather soft, indolent tumor corresponding to the lower part of the right thyroid lobe

showed hemoglobin (Hgb) 13 4 g/100 ml SR 22 mm per hour white blood cell count 3400 serum creatinin 1 0 mg/100 ml Three days after admission the thyroid gland was exposed via a collar incision, the right recurrent nerve was isolated and a hemithyroidectomy including the tumor was *performed on the right side*

The histopathological finding was a radically extirpated metastasis from a renal carcinoma to the thyroid. It was classed as malignancy grade II A same as the primary tumor

At the last physical examination in August 1967 there were no signs of recurrence of the tumor or further metastases

Case 2

The patient was a 46-year-old man In December 1966 he noted pain in the groin Urography in January 1967 showed an expansive process in the right kidney and renal angiography demonstrated a densely vascularized tumor (*Fig* 3) *No metastases could be detected, and the left kidney* was roentgenographically normal The SR was 66 mm hour and Hgb 10 3 g/100 ml A needle-biopsy from the tumor was attempted but no tumor cells were obtained

Nephrectomy on the right side was performed in March 1967 The upper pole of the kidney was occupied by a grapefruit sized tumor which was



Fig. 8. Larger magnific tion of the metastasis show in Fig. 7. The cellular pattern is less polymorphic tha that f the primary t mor in Fig. 5, t which it should be compared. *H E* 160.

excised. The pathological examination showed a totally extirpated metastasis from a renal adenocarcinoma. *It was more highly differentiated than* the primary tumor which explaines the discrepancy between the cytological picture (high differentiation) and the histopathological one ("medium high differentiation") The thyroid parenchyma was otherwise normal (Figs. and 8)

Three months after the operation on the thyroid (8 months after the nephrect my) there were no signs of recurrence r further metastasis.

Case 3

This male patient was 38 years old, when, in 1938, he had an acute episode of abdominal pain and blood in the urine. A fist sized mass could be pal pated in the region of the left kidney No other examinations are recorded The patient was operated on in October 1938 with a nephrectomy on the right side The kidn y was partially occupied by an orange-sized tumor which was totally *removed. No metastases could be detected.*

Th microscopic slides showed a renal adenocarcinoma of malignancy grade II B, without signs of penetration of the renal pelvis or vessels.

The patient remained well and without ymptoms until 1950, when he



Fig 4



Fig 5.

Fig. 4 Microphotogram of the primary tumo of case 2, howing the structure and cellular pi ture of a renal ad nocarcinoma, malignancy grad II B. Ha matoxylin Eosin ×160

Fig. 5 Mi roph togram f th metastasis to the thyroid gland in case 2. In th pper part of the pitcure is seen thyroid parenchyma in th lower part neoplastic tissu H E. ×64

In May 1967 irradiation to the previously operated area was started, but when 4500 r had ben given the platelets had decreased to 60 000/cc and irradiation was temporarily discontinued During the period of irradiation repeated urographies of the left kidney and X rays of the lungs had been normal

The patient was admitted to the Department of Otolaryngology in August 1967 for surgical exploration of the thyroid region Physical examination revealed a walnut sized, hard tumor in the right thyroid lobe and laboratory examination showed Hgb 11 2 g/100 ml SR 58 mm/hour white blood count 2700/cc with 42% lymphocytes platelet count 85 000/cc. As the cytologic diagnosis had not been clear-cut, the surgical intervention on the thyroid gland was as much a diagnostic as a therapeutic one

At operation the thyroid gland was exposed via a collar incision and as the tumor was well circumscribed from the thyroid tissue, it was easily

DISCUSSION

Incidence of Metastases to the Thyroid Gland

The incidence of clinically manifest metastasis to the thyroid gland is much lower than that found in series based on autopsy material.[1] This is to be expected, as the autopsy cases usually present the neoplastic disease in a more advanced stage. Thus, in a series of 30 000 thyroidectomies performed in the Mayo clinic from 1892 to 1945 Long & Black (1945) reported 22 instances of carcinomatous metastasis to the thyroid gland (three of these were from renal adenocarcinomas) and Bruce & Michie (1954) in a series of 1600 thyroidectomies, reported four instances of secondaries (two of these were from renal adenocarcinomas). Series based on autopsies, however, show that the thyroid gland is more frequently the site of metastasis than is usually recognized. In consecutive autopsies on patients with unselected primary cancers, Willis (1931) reported nine instances of metastasis to the thyroid gland out of 170 cases (5.3%), Mortensen *et al* (1956) 18 out of 467 cases (3.9%) and Shimaoka *et al* (1962) 188 in 1999 cases (9.4). In this last series only nine cases of primary thyroid carcinomas were found, which seems to indicate that in patients with cancer of another organ, metastasis to the thyroid gland occurs about 20 times more frequently than primary thyroid carcinoma.

Most of the reported metastases to the thyroid have occurred in abnormal glands, usually a colloid goiter. This experience is supported by experimental results, because an abnormality which decreases the blood-flow through an organ can enhance the formation of metastasis in two ways. (1) The slow blood-flow facilitates the deposition of tumor cells according to Coman *et al* (1951) and Fischer *et al* (1967) and (2) a lowered oxygen tension favours the growth of anaerobic i.e. tumor cells. (Warburg, 1926 Bruce & Michie 1954 Lange 1960). In two of our cases the metastasis occurred in abnormal thyroid tissue (cases 3 and 4).

Diagnosis

Even if as pointed out by Shimaoka (Shimaoka *et al* 1962) a malignant tumor in the thyroid gland in a patient known to have another cancer is more likely to be metastatic than primary, the possibility that it is a second primary neoplasm should always be considered. Also, in the case where no primary tumor is known, a thyroid nodule could represent a metastasis from an undetected cancer. The diagnosis of thyroid tumors is therefore very important and as much information as possible should be gathered preoperatively, for example by I[131]-uptake scintigram and needle biopsy (Einhorn & Franzén, 1962). The cytological differential diagnosis, however, be

[1] Apparently this is not valid for renal adenocarcinoma as stated in the introduction of this paper.

noticed a swelling of the thyroid He had no signs of toxicity and a consulted doctor diagnosed goiter As the swelling did not bother the patient, it was decided to leave the thyroid untreated But the gland continued to enlarge, and in April 1953 a partial strumectomy was attempted The thyroid and its surroundings turned out to be the seat of tumor growth, however and it was not technically possible to remove the neoplasia completely Microscopy revealed metastatic renal adenocarcinoma of malignancy grade II A to a colloid goiter

The patient was referred to Radiumhemmet for post-operative irradiation After this treatment the patient remained well and without signs of further metastases until November 1953 when a secondary in the right tibia was discovered This regressed after radiation and no progression of the disease was noticed until February 1956 when cranial nerve signs developed. The patient died in December the same year with widespread cerebral metastases No autopsy was performed.

Case 4

The patient was a male, who at the time of diagnosis was 66 years old Since "many years he had a slowly increasing enlargement of the thyroid gland without signs of toxicity The lesion had been diagnosed as a non toxic goiter and no treatment had been given

In February 1962 the patient noted dark-coloured urine Laboratory examinations showed Hgb 14 mg/100 ml SR 15 mm/hour Renal angiography demonstrated a tumor in the left kidney and also a probable metastasis in the left adrenal Nephrectomy was performed in February 1962, but the adrenal was not extirpated The tumor was a renal adenocarcinoma of malignancy grade II A and had been radically excised.

Nine months later the patient was again admitted to the hospital because of compression symptoms from the enlarged thyroid gland The gland had just before the admission started to grow rapidly and was now interfearing with the patient s breathing X rays indicated a grossly enlarged and partially intra thoracical thyroid I^{131} tracing gave normal values, but the scintigram did not show any uptake in the intra thoracic part. During the laboratory investigations the patient developed acute dyspnea necessitating surgical intervention. At operation several tumors were found in the enormously enlarged thyroid, and numerous large, hard lymph nodes were also discovered As some of the tumor tissue infiltrated in the surroundings, radical removal was not possible

Microscopy showed metastases to a colloid goiter and lymph nodes from an adenocarcinoma with similar structures and malignancy grade as the patient s renal tumor

Post-operatively the patient was referred to Radiumhemmet for irradiation therapy The disease progressed, however and the patient died in October 1964 with generalized metastases He was not autopsied

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tween a thyroid adenoma, a well differentiated thyroid carcinoma and a metastasis from some other well differentiated adenocarcinoma can be utterly difficult (as was demonstrated in our case no 2) Frozen sections during operation can also in the above-mentioned cases, be extremely hard to evaluate (Burge & Blalock, 1967)

Treatment

The principle of removing an organ because of the presence of a metastasis should be applied also to the thyroid, and especially if the primary carcinoma appears to be controlled and there is no evidence of metastasis elsewhere In the case of a single metastasis simple extirpation seems justified, whereas in the case of multiple metastases, general principles for surgery in thyroid cancers should be applied the exception being, however that radical neck-dissection seldom is indicated. Post-operative irradiation should be considered in the cases where radical removal of tumorous tissue can not be accomplished

Clinical Course

The clinical course of renal adenocarcinoma is relatively often one of slow progression with late development of metastases In the cases with secondaries to the thyroid, for example, the interval between the detection of the primary tumor and the development of clinical manifest metastasis averaged $9^1/_2$ years in the 18 patients, where it was recorded (range 3–23 years) [1] In two of our cases the metastases developed shortly after nephrectomy but in the other two there was a free interval of 12 and 16 years, respectively Such long intervals illustrate one of the bizarre characters of renal adenocarcinoma, which it sometimes shares with some other and usually hormone-dependent neoplasias (Morton & Morton, 1953) These free intervals have led to the theory of dormant cells" (Hadfield, 1954) which have received some experimental support (Gardner 1945 Fischer & Fischer 1950) Our two cases were not autopsied, however and a new renal adenocarcinoma in the remaining kidney can therefore not be excluded.

ZUSAMMENFASSUNG

Es sind vier Fälle mit Adenokarzinom der Niere mit Metastasen in der Schilddrüse gemeldet. In zwei Fällen entwickelten sich die Metastasen gleichzeitig mit dem Primartumor und in den anderen zwei Fällen bestand ein Intervall von 12 bzw 16 Jahren von der Nephrectomie bis zum Auftreten der Metastasen. Einige der bizarren Kennzeichen des renalen Adenokarzinoms sind kurz diskutiert worden ebenso das Vorkommen von Meta tasen in der Schilddrüse im Allgemeinen ihre Diagnose und die Prinzipien der Behandlung Diese vier Fälle bringen die totale Anzahl der veröffentlichten Fälle von Adenokarzinom der Nieren mit Metastasen in der Schilddrüse, die wir in der Literatur gefunden haben, auf insgesamt 48

[1] Figure calcul ted by the uthors from th published papers.

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ELECTRON MICROSCOPIC STUDIES OF THE OTOSCLEROTIC FOCUS

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From electron microscopic studies of otosclerotic tissue fixed immediately after surgical removal of the stapes, we feel we can deduce the following. First: Apart from the borderline between focus and normal tissue as seen in the light microscope, there are "microfoci" demonstrable only in the electron microscope. The focus appears to enlarge by fusion of these microfoci. Second: Osteoclasts appear to play a subordinate role during the so-called phase of resorption. On the other hand, we demonstrated pronounced osteocytic resorption, pericellular lysis of collagen being an invariable finding. In relation to these findings, lysosomes were often observed in the osteocytes. The endothelial cells of the capillary network appeared to be normal.

Otosclerosis has not as yet been subjected to thorough study by electron microscopy, only a few investigations by this method being on record (Chevance 1962, Reydon & Smith, 1968, Frank *et al.* 1968). This fact is surprising, considering the gaps in our knowledge of this disease, especially its cytological aspects. To-day there is relatively ample material for study since surgical removal of the stapes is now practised in most E.N.T. departments. However, the explanation of this lack of investigation refers to certain difficulties concerning the properties of the material.

In the first place, the demand for immediate fixation of the removed bony fragment requires a close collaboration between surgeon and cytologist. The surgeon must also indicate where in the stapes or in the removed bony fragment the focus is situated. Lacking a precise orientation of the material during the embedding process, the focus may easily be lost.

Another and perhaps greater difficulty is that the tissue to be studied is bone, and without decalcification it is not possible to obtain sections thin enough for cytological study. Without decalcification the presence of calcium would prevent the electrons from penetrating the material.

Chevance (1960) suggested using EDTA (ethylene diamin tetraacetic acid) ... decalcification medium which was applicable to the bony labyrinth at ... neutral pH. This chelating substance was used in the present study ... acid decalcification media are not applicable in electron microscopic studies. The technique will be briefly described below.

The stapes or fragment of the stapes, and in some cases osteoid lamellae removed at operation for otosclerosis in Béziers and in Copenhagen were

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Fig. 1. Electron micrograph showing "osteoid lamellae" (*OL*)—small new formation of bone lamella surrounding the osteocytes (*O*). These bony structures are located under the mucosa (fibroblasts, *F*). × 8000

Fig. 2. Electron micrograph showing "macrofocus" (*MF*) with absence of collagen fibers in newly formed bones. × 23,000

fixed immediately after removal in a solution of 25% glutaraldehyde mixed with cacodylate buffer pH 7.2.

After 24 hours at 4 C in this solution the preparations were decalcified in a 4.5% solution of EDTA 2 Na adjusted by NaOH to pH 7.2. The decalcification in this solution at 4 C takes 2–4 days. The decalcified preparations may be stored in the cacodylate buffer for several weeks.

After rinsing in Tyrode's solution and post fixation in 4% osmic acid, OsO_4, the preparations were dehydrated and embedded in Epon as advocated by Luft (1961). Epon solution A and B is mixed at the ratio 7 : 3.

From the preparation blocks, survey sections $^1/_2$–1 μ thick were made for light microscopic studies. These sections were stained with 1% toluidine blue in a saturated solution of borax. Thereafter small pyramids were made from the transitional zone between otosclerotic and normal bony tissue. Ultrathin sections of these pyramids were collected on coated copper grids and contrasted with uranyl acetate and basic Pb citrate for 1–5 min at room temperature.

The electron microscopic studies were performed in Paris with a type Hitachi microscope and in Copenhagen with a Philips 200.

RESULTS

As the pictures obtained by these technical procedures show certain changes, the point is (as in all electron microscopic studies of abnormal tissues) to make sure that such changes are not artefacts due mainly to the process of decalcification but really represent the cytological appearance of the otosclerotic focus.

This very important point was solved by a close collaboration between
The electron microscopic studies were performed in Paris with a type
surgeon and cytologist. Causse (1960) has described as "osteoid lamellae" small new formations of bony lammellae found in cases of developing otosclerosis in the very limited area between the fissula ante fenestram and the anterior pole of the stapes. These very thin bony structures are arranged like the tiles on a roof beneath the mucosa of the abovementioned zone. They seem to creep from the fissula to the stapes which they eventually surround and block.

We first studied these abnormal structures which are, if not constant then very common phenomena during the development of the disease. They always present themselves as new developing otosclerotic bone so thin and so loosely bound to their organic matrix that they can be cut without decalcification, unlike the hard and brittle bone of the stapes. Accordingly these "osteoid lammellae" may be considered perfect reference material in comparing with the decalcified stapedial bone (Fig. 1).

The electron microscopic studies of the otosclerotic focus may conveniently be divided into two parts: (a) the bony tissue; (b) the cells.



FIG. 1 Electron micrograph showing osteoid lamellae (*OL*)—small new formations of bone lamellae, surrounding the osteocytes (*O*). These bone structures are located under the masses of fibroblasts, *F*) 8000

FIG. 2. Electron micrograph showing "microfocus" (*MF*) with absence of collagen fibers in newly formed bones. 33,000.

a

b



Fig. 1. ( ) Electron micrograph sh wing osteocyte (*O*) i its lacuna on the way of lysis. Not the rarefactio of the normal cell lar organelles, especially the lack of mitochondria. The cell i shrunken. 14,000 (*b*) Electron micrograph showing more adv nce stage in the lysis of the osteocytes (*O*) The cell is shru ken and its borde (*B*) is no more in contact with the border of the lacuna. 20,500. ( ) Electro micrograph showing an osteocyte undergoing lysis with nclear fragmentati and cyt plasmatic vacu lisation. 20,000.

The bony tissue

After decalcification, this tissue presents itself as being made up mainly of a multitude of interwoven collagenous bundles, so entangled and intricate that a systematic description is impossible. It is in fact what the German histologists called Faser knochen No other bone shows such strong collagenous matrix. It is not possible to distinguish anything in the way of a Haversian system. Two facts should be emphasized

1 Cartilage cells are never found within the limits of an otosclerotic focus.

2. A striking finding in the collagen tissue, not suspected from the light microscopic studies, is a multitude of "microfoci" in the immediate neighbourhood of the large more evident ones (Fig 2) The borderline between normal and diseased tissue is always very sharp with no fringe in the periphery of the foci, not even at high magnification.

The entire pictures gi es the impression that the disease is splashing" around from active centres.



Fig 4 Electron micrograph showing a normal aspect of a capillary (C) running through an otosclerotic focus (OF) 15,000

Regardless of their size, the foci generally present themselves as gaps or empty zones, but sometimes the process of destruction is not complete and the collagen bundles may be observed in the process of lysis. The individual bundle gets thinner losing its normal periodic striation at some distance from the end of the fibre

The cells

The cellular aspects are not so uniform lysis predominating Not uncommonly there are lacunae in the tissue outlining the general shape and size of an osteocyte which has totally disappeared This finding has to be studied as a function of the surrounding tissue If it seems normal the finding may be interpreted as the remnant of an osteocyte whose lacuna has filled with a labile calcium deposit which has disappeared in the course of decalcification Such a finding is not of immediate pathological significance

On the other hand, if the surrounding tissue shows one or more active otosclerotic foci in the immediate vicinity the lytic process may be considered an active one If so however the surrounding collagenous structures display discrete signs of changes, chiefly in the form of localized rarefaction or irregularity of the cell lacuna

More interest attaches to the finding of cells in the process of lysis (Fig 3 a b c) The first stage is loss or rarefaction of the normal cellular



Fig. 3. Electron micrograph showi g an osteocyt with it ucleu (N) I the yt plasm umber of round esicles f differ t si es containing osmi philic mat rial (lysosomes) are prominent features (L) x 10,000

organelles The m st striking feature is rarefaction of the mitochondria and shrinkage f the cell, its borders being no longer anywhere in contact with the borders of its lacuna. The second stage is partial lysis, nuclear fragmentation and cytoplasmic acuolization

These appearances call to mind first the possibility of artefacts, but for two reasons w believ that they do not represent artefacts

(a) Many times we ha e observed, in the same electron microscopic pic ture a normal cell and nearby a cell in the process of lysis, and an artefact due to fixation cannot affect one cell and not another only about 50 square µ apart

(b) Osteoid lamellae which we hav studied without decalcification ha e shown essentially the sam process of lysis.

These findings re highly suggestive of an initial role played by the cells in otosclerosis. Incidentally our findings ha e not confirmed the theory advanced by some uthors that the capillaries are important factors in the genesi and spread of the disease Fig 4 shows a perfectly n rmal capillary running through an active otoscleroti focus.

It is striking that we have nev r made any findings which might uggest an active osteoclastic ell.

Of course, a negati e finding alway ha to be regarded with caution in lectron microscopy a the field is v ry limited, even when th usands of sections hav been studied as in our investigation N ve theless, thi nega-

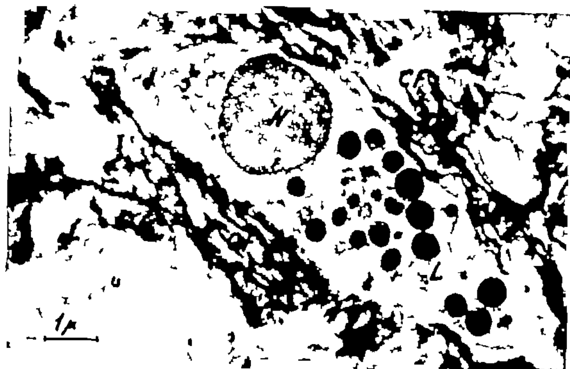


Fig 6 Electron micrograph showing the same as Fig. 5. Note the pericellular surrounding matrix with few collagen fibers (*CF*) ×19 000

tive finding indicates that osteoclastic bony resorption cannot be considered as the usual process in otosclerosis And even though osteoclastic absorption did occur it could not explain the greater part of the pathological processes, cf the spread and variety of the resorption foci On the other hand, the large number of osteocytes surrounding the foci exhibit certain morphological peculiarities which may be considered of the utmost importance to the understanding of otosclerosis.

These abnormal osteocytes show a number of rounded cytoplasmic vesicles of different size, surrounded by a one-layered limiting membrane. These cellular organelles we have interpreted as lysosomes (Figs 5 and 6) Lysosomes, first described by Duve (1963) are attributed with a large number of enzymatic activities, so far about 15 different ones. Most of these enzymes are hydrolytic Thus, among other activities, they are able to dissociate and dissolve protein structures, even as resistant as collagen Lysosomes were first found in the cytoplasm of liver cells They occur in a wide variety of cells, but have never been described in normal osteocytes. One of the first enzymes reported to be lysosomal was acid phosphatase, and its presence is still considered by many to be a good sign of lysosomal activity In 1961 Albernaz & Covell reported the presence of this acid phosphatase in active otosclerotic foci Since then, it has been found by us and many others in active otosclerotic foci The important role of lysosomes in the resorption of bone which characterizes the initial stage of otosclerosis is in agreement with certain findings of physiological resorption of bone Vaes

(1965) has demonstrated release of lysosomal enzymes in culture medium from bone cells undergoing resorption due to the action of the parathyroid hormone.

Moreover it has recently been emphasized by Bélanger (1965) that there might be cases of what he called osteocytic resorption without osteoclastic intervention. We believe that this is what largely happens in otosclerosis.

It should not be inferred from our description that lysosomes are very commonly encountered in electron microscopic studies. The reason why they are not is probably that as soon as the lysosomes grow and liberate their enzymes, the cell will undergo rapid lysis. Therefore, it may be expected, statistically that a large number of sections have to be studied before coming upon cells in the very process of lysis.

What in our opinion may throw light upon the entire process of lysis in otosclerosis is a finding in cultures of embryonic bone cells and the common knowledge that embryonic remnants abound in the fissula ante fenestram, *generally considered as the zone of its initial spread. The finding to* which we are referring is that lysosomal lytic activity may be increased by oxygenating the culture perhaps only by a few per cent above the normal partial pressures of oxygen in the culture medium (Sledge, 1965)

It is well-known that 80 years ago Schwartze described marked hyperaemia as a constant sign of active otosclerotic foci. Hyperaemia means a greater amount of oxygen to the diseased cells, and this may explain the swelling and rupture of lysosomal vesicles responsible for the lytic process. Cinca, of Bucharest, recently stated that as many as 62 theories aimed at explaining otosclerosis by looking for the causal factor of the disease. Our opinion is quite different. We believe that the search for a cause, or even causes, will lead nowhere. We do not offer a new theory We want to settle the problem on the cytological and cytochemical level, and we feel sure that this will prove a *more fruitful way of research.*

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ZUSAMMENFASSUNG

Bei elektronenmikroskopischen Untersuchungen otosclerotisch m Gewebe, das unmittelbar nach Operationen dieser Krankheit fix ert ist, haben wir folgende Resultate gefunden. Erstens Ausserhalb *der beobacht ten Grenzlinl* des Lichtmikroskopes findet man, zwischen F kus und normalem Gewebe, „microfoci die man nur im Elektronenmikroskop beobachten ka n. Fokus scheint bei ei er Verschmelzung dieser microfoc zu wachsen. Zwe tens Osteoclasten scheint nur

eine untergeordnete Rolle der sogenannten Resorptionsphase zu spielen. Dagegen haben wir eine ausgesprochene osteocytische Resorption nachgewiesen als peri cellulär Lysis von Collagen regelmässig gefunden sind. Im Verhaltnis dazu findet man manchmal Lysosomer in den Osteocyten. Die Endothelzellen der Kapillaren scheinen normal.

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GLYCOGEN IN THE INNER EAR AFTER ACOUSTIC STIMULATION

A light and electron microscopic study

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Glycogen was locali ed the inner ear of arious spe ies at the light ad electron microscopic le L Sev ral tech iques of ti sue fixati n and embeddi g were tested. Light microscopi demonstrat on of glycogen was consistent and ptimal in gl taral leh de-f ed and Epon-embedded spec mens stai ed w th the period acid Schiff reaction. With this sta dard method, well-d fined intracytoplasmic glycogen granules were red t purple W th th p riodic cid-thiosemicarbazide method for lec tron microscopi demonstrati n of glycogen numerous isodi metric lectron-dense part cles were seen n the same cell regions in whi h the PAS-positi gra ules were bserved These electro -dense gra ules w re digestible w th α-am lase.

The d stribution pattern sh wed marked spec es differen es In gui e p gs glycog n gr nules were limited to the ter hair cells of th coch-lea w th a decrease basalwards. In mice most of the glycogen granules were seen in De ters cells and less in th outer hair c lls. In cats, no gly ge could be demonstrated. In no e of these spec es was glycogen observed i th estibular sensory and supporting cells.

After e posing guinea pigs t 110 dB white n ise for 30 minutes, there w quantitati e decre se of glycogen. Three hours after acousti stimulation umerous smalle granules were detect ble nd in 12 to 24 hours these aggreg ted t form bigger granules. Finally 24 hours after the posure t oise th d stribution pattern of glycogen revert d to normal. Th h ges f llow ng acoustic stimulatio may be due to glyco-g nol si nd onsecuti gl cogenesis, suggesting that glycogen serves a energy source n th hai cells.

Gl cogen, $(C_6H\ O_5)_n$ i polysaccharide made up from glucose units and distributed through the cyt pl m a a source f en rgy via glycogeno-ly i Consid ring the blood su ply of the rgan of Corti, anaerobic glyco-lysi may hav n important role in its metabolism with glycogen as the ma n source of ne gy Alth ugh sev ral investigators have studied the

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TABLE 1 *Review of light microscopic studies*

Species	Authors	Localization: Inner hair cells	Outer hair cells	Dieters cells	Hansen's cells	Remarks
Guinea pig	Zorzoli 1951	+ +	+ +			
	Vinnikov & Titova 1957	−	+ +			surface (flat) specimen
	Finzi, 1958	−	+ +			
	Falbe-Hansen & Thomsen, 1963	−	+ +			
	Present authors	−	+ +			
Mouse	Present authors	−	±	+ +	±	
Bat (hibernating)	Plotz & Perlman, 1955	+	−	+ +		freeze-dried specimen
Rat	Bélanger 1956	−	+ +	+		
Rabbit	Vinnikov & Titova, 1957	+ +	+ +	+ +	+ +	surface (flat) specimen
Cat	Vinnikov & Titova, 1957	−	+	+ +	+ +	surface (flat) specimen
	Falbe-Hansen & Thomsen 1963	−	−	−	−	
	Present authors	−	−	−	−	

− glycog n n t demonstrabl ± little or no glycogen + moderate amount of glycogen + + rich in glycogen glycogen decreasing basalwards.

distribution of glycogen in normal and acoustically traumatized animals, there appears to be a great discrepancy among the published results (see Table 1)

Therefore we have compared the results of various histochemical techniques and identified glycogen with the light and electron microscope in normal animals In order to clarify the controversy about the effects of acoustic stimulation on glycogen in the organ of Corti, we have also exposed guinea pigs to white noise In this publication we report the results of these studies

MATERIAL AND METHODS

Light microscopy

Ten young albino guinea pigs, two young cats and two young albino mice were sacrificed under light anaesthesia with ether or pentobarbital sodium Their temporal bones were removed, trimmed stapedectomized and fixed with Lillie's picric acid alcohol (4 C) for 24 hours or in 2.5% buffered glutaraldehyde (4 C) for 20 minutes They were then dehydrated with alcohol and embedded in Epon mixture



Fig. 1 Glycogen i the organ of Corti of normal guinea pigs. ( ) Api 1 turn, (b) 3rd turn, ( ) 2nd turn, (d) basal t rn. The amount of glycoge decre ses towards the basal turn. Glutaraldehyde-fixed, Epon-embedded tissues tained w th the periodic acid-Schiff (PAS) reaction ( ) Freeze-dried, Epon-embedded tissu ; PAS reaction. Be ide the granular ppearance there is diffuse cytoplasmic stain. (f Glycog granules ppe more delicat th Sel gman's method. All photomicrographs were take at the same magnific tion.

Two temporal bones of guinea pigs were dropped in isopentane chilled in liquid nitrogen, followed by freeze-drying with a Speedivac Pearse tissue dryer Model 1 fixed overnight at 65 C in the vapor phase of formaldehyde and directly embedded in Epon mixture.

Epon blocks were cut at 3 μ with glass knives using a Porter Blum ultramicrotome. Sections were picked up with a drop of water and slightly warmed to stick firmly on cover slips. Glycogen was demonstrated with the periodic acid Schiff (PAS) reaction (MacManus, 1948) or with Seligman's periodic acid-thiosemicarbazide technique (Seligman *et al* 1965) For both methods sections were oxidized for 90 minutes in a 0.5% solution of periodic acid and kept for another 90 minutes in the respective reagent solution. In the case of Seligman's method, the sections were placed in a 1% buffered osmium tetroxide solution for 30 minutes. *In some instances* glycogen was digested with a 1% α-amylase solution (buffered to pH 6.0) prior to staining All sections were dehydrated and mounted with synthetic mounting media.

Electron microscopy

Two temporal bones of guinea pigs were processed for electron microscopic studies. Tissues were fixed and embedded as for light microscopy and ultrathin sections were cut with a diamond knife on an LKB ultratome Section picked up on gold grids were treated with 0.5% periodic acid solution for 45 minutes, then for 45 minutes in a 1% thiosemicarbazide solution (the solvent was 25% acetic acid in 70% alcohol) afterwards kept in a 1% buffered osmium tetroxide for 30 minutes and finally stained with uranyl acetate and lead citrate For enzymatic digestion of glycogen, sections were incubated (37 C) with a 1% buffered α-amylase solution for 45 minutes prior to staining (Rosa & Johnson, 1967) Specimens were then examined and photographed under a Siemens Elmiskop 1

Acoustic trauma

Fifteen young albino guinea pigs were exposed for 30 minutes to 110 dB white noise by reference to 0.0002 dyne cm² in a free field. Animals in groups of three were sacrificed 0 3, 6, 12 and 24 hours after the noise exposure and cochleas processed for light microscopy

RESULTS

Normal Animals

Light microscopy

Tissues were better preserved with 2.5 glutaraldehyde fixation than with Lillie's picric acid alcohol which produced marked *shrinkage of the* hair cells. In glutaraldehyde fixed cochleas, glycogen granules were stained magenta red with the PAS reaction (Fig. 1 a–d) In tissues digested with α-amylase before staining, the reaction was not completely negative but

Fig. 3 Electron micrographs of g p g orga f Corti stained with the Seligman method for glycogen. A Two outer hair cell HC contai numerous aryl pl mle glycogen granules that appear black. 8000. B Specimen digested with amylase before Seligman reaction. The distribution of the intracytoplasmic pore corresponds to that of the glycogen granules. The nuclei of two outer hair cells HC and the cytoplasm of supporting cells D did not contain digestible material. 6300

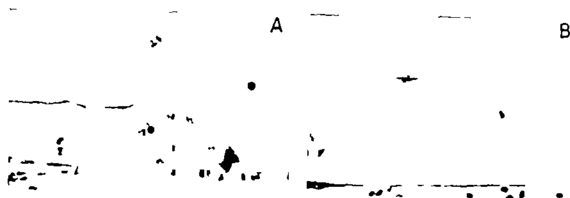


Fig. 2. Striking species differences are evident: there is abundant glycogen in the supporting cells of mice (A) but it is not demonstrable in cats (B). Glutaraldehyde-fixed, Epon-embedded, PAS-stained tissues from upper turn of cochlea. × 330

became much weaker. In comparison, freeze-dried tissues showed considerable variation in tissue preservation: frequently the hair cells appeared shrunken. However, the PAS reaction was strongly positive throughout the cytoplasm of the shriveled outer hair cells, perhaps due to the concentration of glycogen. Better preserved hair cells showed a fine reticular pattern of PAS-positive material (Fig. 1 *e*). There was some difference in the distribution of glycogen using the PAS or Seligman's methods: the latter showed more numerous and finer granules in the cytoplasm of the outer hair cells (Fig. 1 *f*).

Marked species differences were noted. In *guinea pigs* the outer hair cells of the apical area were very rich in glycogen, but the reaction gradually decreased towards the base until no PAS-positive material was demonstrable in the lower half of the basal turn (Fig. 1 *a–d*). The inner hair cells and the vestibular sensory cells were almost completely without glycogen granules. In *mice* glycogen was abundant in the supporting cells, especially in Deiters' cells, whereas the outer hair cells contained only a few granules (Fig. 2 *A*). There were no appreciable differences between various turns of the cochlea, as in guinea pigs. In *cats* glycogen was not demonstrable with either histochemical method (Fig. 2 *B*).

Electron microscopy

Ultrathin sections incubated with Seligman's method contained numerous well defined black granules throughout the cytoplasm of the outer hair cells (Fig. 3 *A*). The granules were roughly isodiametric and appeared larger than unstained glycogen particles. The great majority of these granules were missing in specimens digested with α-amylase. On the other hand, numerous round or oval clear spaces were seen in such sections. The distribution of these spaces corresponded to that of the glycogen granules (Fig. 3 *B*). Supporting cells, nerve endings and inner hair cells showed only sparse granules.

wards the size of the granules decreased but they were more numerous than normal. Between 12 and 24 hours after exposure, the granules aggregated and appeared bigger although less numerous. Finally 24 hours after the noise exposure, the glycogen granules in the outer hair cells appeared nor mal in most of the cases (Fig 4 A–D) *Despite this general tendency* however no appreciable changes could be detected in a few animals.

DISCUSSION

Several histochemical methods have been recommended for the light and electron microscopic localization of glycogen. The specificity of all these methods ultimately depends on the enzymatic *digestion of the demonstrated* substance. For light microscopy the PAS reaction controlled by enzymatic digestion is one of the most commonly used techniques, even though it has some disadvantages. F r instance enzymatic digestion may be incomplete in thick histological sections, or glycogen granules may be resistant to digestion in fixed tissues. Another problem is due to the water solubility of glycogen, which may partly elude demonstration with aqueous reagents. Of course there remains a possibility that protein-bound glycogen (desmoglycogen) is not demonstrated with these histochemical methods (Kugler & Wilkinson, 1960) Watson (1958) used lead citrate staining for electron microscopic localization of glycogen after glutaraldehyde and or osmium tetroxide fixation with this method glycogen appears as approximately isodiametric particles (β-particles) or their aggregates (α-particles) Seligman and co-workers in 1965 established a new method using periodic acid oxidation and a group of chemicals such as thiocarbohydrazide or thiosemicarbazide followed with post-osmication, which theoretically seems more specific than lead citrate staining Rosa & J hnson (1967) have recently been *able to digest* glycogen particles in *Epon-embedded ultrathin sections*, indicating that the specificity of the electron microscopic observations can be checked. At the present, this seems to be the most reliable electron microscopic technique for the visualization of glycogen because the digestion pattern is rather distinct and consistent

The inner ear present special technical problems for the histochemical demonstration of glycogen At the present, bones cannot be decalcified without loss f glycogen (Trott *et al* 1962) th refore the cochlea must be sectioned without decalcification. Another though less formidable, obstacle is proper fixation. It is true that alcoholic fixatives will preserve glycogen for light microscopy but they produce several artifacts such as cell shrinkage intracellular "polarization ("Alkoholflucht) of glycogen etc. Both phenomena are quite triking in the organ of Corti, especially in the outer hair cells thi is understandable because the hair cells are directly exposed to fixatives. We obtained satisfact ry fixation and preservation of glycogen with short-term fixation in cold glutaraldehyde followed by embedding in

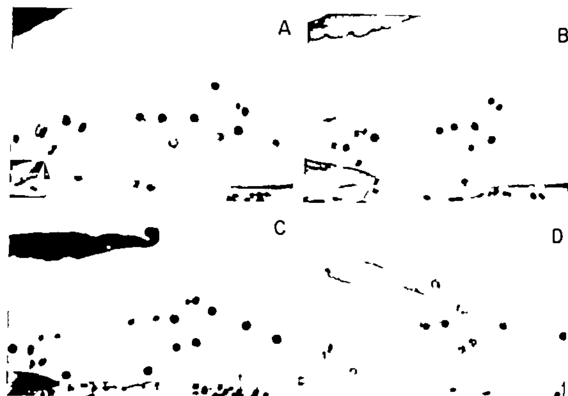


FIG. 4 Effect f acoustic stimulation on glycogen content f outer hair cell f guinea pigs. (*A*) Normal nimal intracytoplasmic glycogen i finely granula (*B*) Three h urs ft exposure t white n ise (110 dB f 30 min) Glycog n c nt nt is dec ased (*C*) Six hours fter exposure t white noise Gly gen granules are ry mall but q ite numerous. (*D*) Twelve h urs after e posure to white n ise. Glycogen gran les re larger nd les numerou than normal. All photographs re from th rgan f Corti at the low 2nd turn Glutaraldehyde fixed, Epon-embedded tissues with the PAS reacti n. × 330

Acoustically Traumatized Animals

The findings in this group were variable, even though care was taken to eliminate possible differences in tissue processing To further minimize variations, observations were made in a standard area, namely 7.5–9 5 mm above the basal end of the cochlea. This specific area can be easily identified, because the secondary osseous spiral lamina is still present but the Boettcher cells are absent[1] Although maximal damage could be expected in this area (Nakamura, 1964) the outer hair cells were missing only in one among 30 cochleas Generally the number of glycogen granules in the examined area decreased up to 3 hours after the acoustic trauma after

W h ml ed th t mpo l bo es f 12 g pig d f nd that I the ag th Boett h ll d th ndary osseou piral lami t nded from the basal d up t 7 43 (t d rd d l tio 0 47) mm a d t 9 30 (0.34) mm, re pecti ly th t tal l gth f th rg of Corti wa 18.33 (0.69) mm

Acc rd g t N k m (1964) aco sti t a m with whit n ise m imal d mag in th 9 6–10 3 mm re f gui pig cochl s. Sinc th t t l l gil f th org n f Corti n hi m t l l w 19 7 ± 2.04 mm, ur bserv t ns in th 7 43–9.30 mm area (f hort org f Corti) are comparabl

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DISCUSSION

Several histochemical methods have been recommended for the light and electron microscopic localization of glycogen. The specificity of all these methods ultimately depends on the enzymatic digestion of the demonstrated substance F r light microscopy the PAS reaction controlled by enzymatic digestion is one of the most commonly used techni ues, even though it has some disadvantages. For instance enzymatic digestion may be incomplete in thick histological sections, or glycogen granules may be resistant to d gestion in fixed tissues. Another problem is due to the water solubility of glycogen, which may partly elude demonstration with aqueous reagents. Of course there remains a possibility that protein-bound glycogen (desmoglycogen) is not demonstrated with these histochemical methods (Kugler & Wilkinson, 1960) Watson (1958) used lead citrate staining for electron microscopic localization of glycogen after glutaraldehyde and or osmium tetroxide fixation with this method glycogen appears as approximately isodiametric particles (β-particles) or their aggregates (α-particles) Seligman and co-workers in 1965 established a new method using periodic acid oxidation and a group of chemicals such as thiocarbohydrazide or thiosemicarbazide followed with post-osmication, which theoretically seems more specific than lead citrate staining. Ross & Johnson (1967) have recently been able t digest glycogen particle in Epon-embedded ultrathin sections, indicating that the pecificity of the electron microscopic observations can be checked. At the present, thi seems to be the most reliable electron microscopic technique for the vi ualization of glycogen because the "digestion pattern i rather distinct and consistent

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Epon This technique was adapted after comparison with freeze-dried specimens in which the loss of glycogen was supposedly minimal A definite answer cannot be given for the incomplete digestion of glycogen with α-amylase. We attributed this to the slow penetration of the enzyme into the relatively thick sections embedded in Epon Evidence for this possibility was provided by the electron microscope which revealed complete digestion of glycogen particles in ultrathin sections. Apparently these technical problems explain in part the contradictory reports on the distribution of glycogen in the cochlea Species differences account for other discrepancies (see also Table 1)

Our observations in guinea pigs and cats confirm the results of Find (1958) and Falbe Hansen & Thomsen (1963) While no data are available on mice to compare with our findings, it is interesting to note that a similar distribution exists in bats (Plotz & Perlman 1955) These marked species differences caution us not to generalize about the metabolic role of glycogen in the cochlea.

The presence of glycogen particles in the organ of Corti of guinea pigs has been noted by several electron microscopists, but these observers have not provided histochemical evidence It was first Vosteen (1964) who specifically demonstrated glycogen in the organ of Corti He used carmine red as a contrast stain for glycogen and found the cytoplasm of the outer hair cells densely packed with carmine positive particles. Moderate amounts were noticed also in the nerve endings and in the inner hair cells. Unfortunately there are no electron microscopic data on the correlation between carmine-positivity and digestion with α-amylase Even with Seligman s method we found undigestible stained particles Therefore, histochemical reactions for glycogen cannot be considered absolutely specific unless confirmed by enzymatic digestion Using this as a relatively reliable technique we demonstrated glycogen exclusively in the outer hair cells of the guinea pig

The findings in acoustically traumatized guinea pigs do not permit definite conclusions about the possible role of glycogen in the metabolism of acoustically traumatized hair cells Nevertheless, it is reasonable to assume that the transient decrease in the number of glycogen granules followed by an increase in smaller granules may be due to glycogenolysis and consecutive glycogenesis. This would imply the enzymatic release of chemical energy after acoustic stimulation and restoration of the depleted glycogen stores by enzymatic process However the marked species differences in the distribution of glycogen indicate that this process is not necessarily operative in the cochlea of all mammals.

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ZUSAMMENFASSUNG

Glykogen wurde mit dem Licht- und Elektronenmikroskop im Innenohr verschiedener Säugetiere dargestellt. Dabei wurden mehrere Fixations- und Einbettungsverfahren geprüft. Der lichtmikroskopische Nachweis von Glykogen war übereinstimmend und optimal mit der Perjodsäure-Schiff'schen (PAS) Reaktion in Präparaten, die in Glutaraldehyd fixiert und in Epon eingebettet wurden. Mit dieser Standardmethode färbten sich die wohlbekannten zytoplasmatische Glykogengranula rot bis violett. Mit der Perjodsäure-Thiosemicarbazid-Methode von Seligman zum elektronenoptischen Nachweis von Glykogen wurden in den gleichen Zellteilen wie die PAS-positive Granula zahlreiche dunkle isodiametrische Partikel beobachtet. Diese elektronendichte Granula konnten mit α-Amylase verdaut werden.

Das Verteilungsmuster wies grosse Unterschiede zwischen verschiedenen Tierarten auf. In Meerschweinchen waren die Glykogenkörnchen auf die äusseren Haarzellen begrenzt und [illegible] nahmen basalwärts ab. In Mäusen wurde Glykogen hauptsächlich in den *Deiters'schen Zellen* beobachtet und weniger in den äusseren Haarzellen. In Katzen konnte kein Glykogen nachgewiesen werden. In keiner der *untersuchten Tierarten war Glykogen in den vestibulären Sinnes-* und Stützzellen [illegible]

Nach Belastung mit *110 dB* white noise *(30 Minuten) nahmen die* Glykogenkörnchen ab. Nach drei Stunden konnte man zahlreiche kleine Granula darstellen, *die sich 12 bis 24 Stunden* nach der *Belastung zu grösseren* Körnchen ansammelten. Nach 24 Stunden war das Verteilungsmuster von Glykogen wieder normal. Die Veränderungen nach dem akustischen Reiz können mit einer Glykolyse und nachfolgendem Glykogenaufbau erklärt werden, und weisen darauf hin, dass Glykogen in den inneren *Haarzellen* von Meerschweinchen eine mögliche *Energiequelle* darstellt.

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SOME MORPHOLOGICAL ASPECTS OF SOUND PERCEPTION IN BIRDS

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The fine structure of the cochlear duct of the cock (Gallus domesticus) was studied by light and electron microscopy. The morphological findings confirmed known physiological facts.

Because of the obvious difficulties associated with studies of the morphology and neurophysiology of the human ear most of the investigations on which our understanding of the function of the peripheral hearing organ is based on other mammals. However, even studies on mammals are not enough to provide a clear picture of the conversion of mechanical energy from the mechanoreceptors to nerve impulses, and comparative morphologic and physiologic research on a wide basis would therefore seem to be essential.

As a link in our investigations of the morphology and function of the mechanoreceptors an attempt has been made in the present study to throw light on the fine structure of the organ of Corti in the cock and to correlate these observations with the known physiologic facts.

The architectonics of the organ of Corti as it is seen under the microscope is familiar from a series of studies by Hasse (1867), Retzius (1884), Held (1926), de Burlet (1934), Stresemann (1934), Kolmer (1928) and others. An excellent survey of the earlier work in this field has been published by Frey (1952). The fine structure of the cochlea in birds has been described by Cordier (1964) and Vinnikov *et al.* (1965).

Light Microscopical Technique

A number of young adult cocks were perfused through the aorta with Heidenhain-Susa solution and the heads subsequently fixed in the same solution, decalcified and bedded in celloidin. The imbedded specimens were then cut in a Leitz cell idin specimen microtome into serial sections with a mean thickness of about 12 μ. The sectioned specimens were stained with haematoxylin and eosin solution and examined with a Zeiss photomicroscope.

Electron Microscopical Technique

Eighteen young adult cocks were anaesthesized deeply with Nembutal and decapitated The temporal bone was dissected and the collumella lifted from the oval window The vestibulum and cochlea were carefully perfused with osmium tetroxide solution (Rhodin, 1954) and the specimen removed and fixed in the same solution During the subsequent dehydration the various parts of the labyrinth were dissected and imbedded separately in Epon 812 (Luft 1961)

For pilot studies about 1 μ thick sections were cut with an LKB ultrotome and stained with toluidine blue solution. After careful orientation of the specimen ultra thin sections were then cut with an LKB ultrotome stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop I Gevaert Scientia 22 D 50 plates were used for photography at an enlargement of between 1000 and 40 000 times.

Light Microscopic Examination

The light microscopic picture of the inner ear of birds has been described by a large number of research workers. It will suffice here therefore to give a short summary of the structure of the membranous labyrinth as a basis for the subsequent detailed description of its fine structure

Development

The basilar papilla or the organ of Corti of birds is developed from the pars inferior of the membraneous labyrinth. As in the turtle in the early stages of development the saccule is still slightly widened to form a small recess, containing the lagena and lacinia The lagena is an area of sensory epithelium covered by otoliths, whereas the lacinia hair cells are in contact with a tectorial membrane

In birds and reptiles this recess is further developed into an elongated tube which, suspended in a cartilaginous frame separates the surrounding perilymphatic fluid into two portions, the scala tympani and the scala vestibuli these are equivalent to those of the higher vertebrates. This membranous tube forms the slightly curved cochlear duct of the bird which contains the organ of Corti, resting on a basement membrane separating the cochlear duct from the scala tympani (Fig 1) The extreme peripheral part of the tube contains the macula lagena, with its otolith-covered sensory cells (Fig 2)

The cochlear duct

The cochlear duct is separated from the scala vestibuli by the tegmentum vasculosum Figs 1 3) This is a membrane consisting of a loose connective tissue, containing a dense capillary network. The richly folded surface which faces the endolymph of the cochlear duct, is covered by an epithelium consisting of light cells and intensely staining dark cells (Fig 3) These two cell types were described by Stresemann (1934) and their presumed secre-

Fig. 1. Light microscopic survey demonstrating the organ of Corti (*OCo*) standing on the vestibular basilar membrane (*BM*) suspended in cartilaginous frame. The anterior ... cartilage (*NC*) on right. The scala vestibuli (*ScV*) is separated from scala media (*ScM*) by vascularized membrane, the tegmentum vasculosum (*TV*). The scala tympani (*ScT*) is below the basilar membrane at the bottom of the picture.

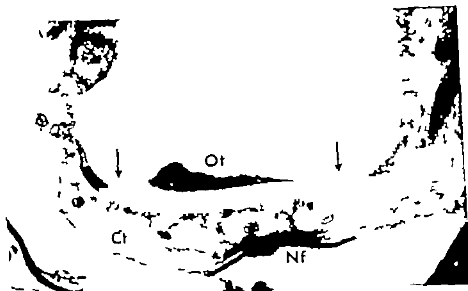


Fig. 2. Macula lagena (arrows) with otoliths (*Ot*). The nerve fibers (*Nf*) are seen penetrating the connective tissue (*Ct*).

Electron Microscopical Technique

Eighteen young adult cocks were anaesthesized deeply with Nembutal and decapitated. The temporal bone was dissected and the collumella lifted from the oval window. The vestibulum and cochlea were carefully perfused with osmium tetroxide solution (Rhodin, 1954) and the specimen removed and fixed in the same solution. During the subsequent dehydration the various parts of the labyrinth were dissected and imbedded separately in Epon 812 (Luft 1961).

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tory function has been studied by among others, Dohlman and co-workers (1959)

Most of the lower tympanal wall of the cochlear duct is occupied by the basilar papilla, a wide vaulted epithelial ridge corresponding to the organ of Corti in mammals. A minor part of the papilla rests on the cartilage capsule while most of it extends over the basilar membrane, which separates the cochlear duct from the scala tympani, and is stretched between the lip-shaped cartilage plates projecting from the anterior and posterior wall in the cartilage capsule (Figs. 1 4)

The basilar membrane can be divided into two regions—an anterior wider zone and a posterior narrower zone The former on which most of the basilar papilla rests, is lenticular in secti n and consists of several layers. Facing the perilymph in the scala tympani is a layer of low cuboidal connec tive tissue cells, which cover the true basilar membrane this is composed of fairly regular parallel fibrils, imbedded, in the fixed specimen, in a fine filamentous matrix. On this membrane rests a layer of irregular connective tissue cells, which forms an extremely loose stratum, poor in cells, and comprising the greater part of the lenticular membrane This layer is divided in its turn from the true epithelium by a thin basal membrane on which the supporting cells of the basilar papilla rest (Fig 4)

Basilar papilla (organ of Corti)

The supporting cells f the basilar papilla are elongated, slightly irregular cylindrical elements, which rest on the basilar membrane and extend up to the surface of the basilar papilla, where they comprise the sensory cells. Under the microscope the cells are light and ha e little structure The basilar papilla is surrounded peripherally by the anterior and posterior hyaline cells (Figs. 1 and 4)

From the anterior ones a fine fibrillar membrane with sometimes hyaline appearance the tectorial membrane projects over the sensory epithelium The membrane surface facing the sensory epithelium is intimately con nected with this through projecting strands that connect the tectorial membrane with the upper side f the upporting cells, so that the membrane forms domes over the individual sensory cells (Fig 4)

Sensory cells

The sensory cells are rod-shaped ranging in length from 8 to 24 μ The section is hexagonal, about 6 μ across. The sensory cells are longest at the top f the curved surface of the sensory pith lium, and diminish in both directions. Each cell gives off a bundle of sensory hairs, which are intimately enclosed in the lower surface of the tect rial membran

N rve fibers

The distal myelinated axons of the cochlear ganglion cells located in the anterior cartilage lamina innervate the sensory cells of the basilar papilla.



FIG. 3 Tegm ntum vascul sum with light cells (*LC*) and dark ells (*DC*) Scala estibuli (ScV) scal media (ScM) Note large capillari s (*ar ws*)

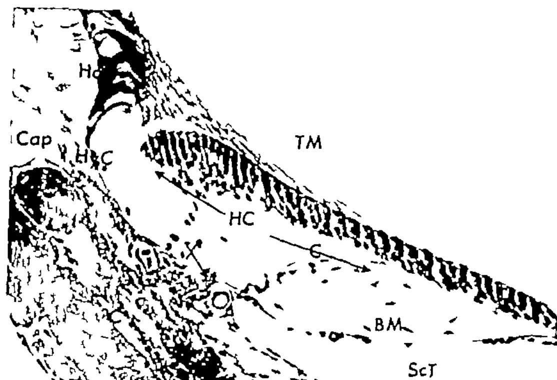


FIG. 4 Detail f th ba la papilla r rg n f Cort w th the tect ri l membrane (*TM*) being u pe ded in a tent haped fashion from the h m ge cell (*H C*) Th h l n cell (*HgC* bel w The ba il r m mbra (*BM*) l tl nl N rv can be seen pe e-trating the rv rtilag (*NC*) d l ing th l myeline h th (*ar ow*) V ry larg capillary l se (*Cap*) Th scala tymp l (*S T*) l t bott m f th picture



Fig. 6. Hair cell top with cuticl (*Cu*) and sensory hairs (*SH*) There is complet ly dev loped kinocilium (*K*) connected t basal body (*BB*) in the part f the cyt plasm free of cuticle. I the posterior part f the basal body basal foot (*BF*) is regularly present. 12,000.

On leaving the cartilage the nerve fibers lose their myeline sheath spread fanwise along the basilar membrane and turn upwards under the sensory cells, at the bottom of which they form nerve endings.

It has been proposed that small unmyelinated nerve fibers innervate the secretory cells of the tegmentum vasculosum (Kolmer 1928)

Electron Microscopy

Sensory hairs and cuticle

The top of the hair cell is partly covered by a dense substance forming the cuticle and located just within the plasma membrane of the flat top of the cell. About 100 sensory hairs protrude from the cuticle (Fig. 5) The posterior part of the hair-cell top has no cuticle in this region there is a basal body or centriole from which protrudes a kinocilium of varying length and development (Fig. 6) The sensory hair which is about 0.2 μ in diameter is composed of a central, fine fibrillar core this is slightly denser at the periphery where it is surrounded by a plasma membrane of the cell (Fig. 7) The axial core of the hairs form a rootlet, which continues into the cuticle The developm nt of the kinocilium is most advanced in the



Fig. 5. The sensory ll re rod sh ped, with d nse reg n th c t le (Cu) l ing only th po t l r p rt free A bundl of se sory ha r (SH) re se n penetrat g the cutl l There kinoc l um, which rl s l appearance (KC) Th t p f th hairs re in close cont t w th the tectorial m mbra e (TM) The u lei (Nu) are ld and located about 5 μ from the top f th hal ll The secret ry ll (SC) xhibit a w t ry cytopl sm where secret ry gr nules" (G) re f u d. 9000

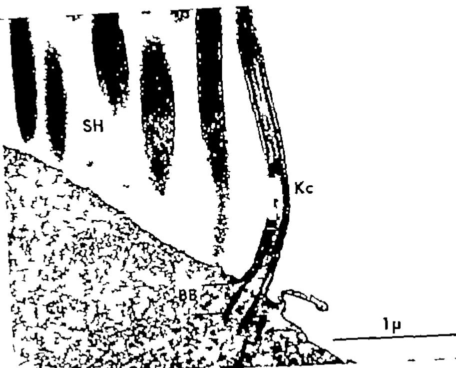


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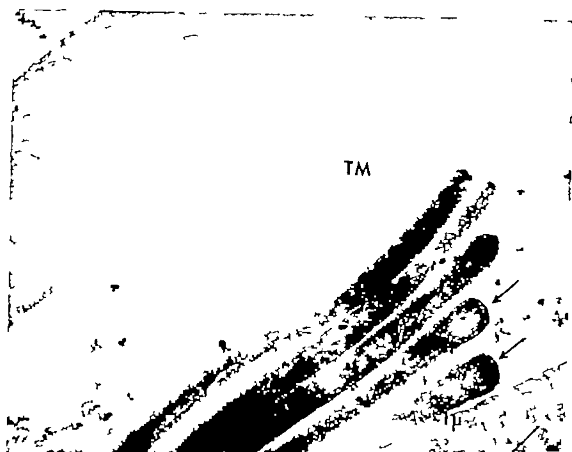


FIG. 7. The sensory hairs are covered by the plasma membrane of the cell and end in a club-shaped swelling (*arrows*). The tectorial membrane (*TM*) adheres itself to the distal part of the hairs, which, compared with mammals, are much bent. ×30,000.

long hair cells in the anterior part of the sensory epithelium. In the anterior short cells the kinocilium is sometimes rudimentary, forming only an extremely short protrusion from the basal body. Each kinocilium proper is composed of a central core with a central fibril bundle composed of nine peripheral double tubular filaments and two central tubular filaments. Each of the former continues down into the cytoplasm of the sensory cell where another short tubular filament is added to, thus forming the centriole or basal body. A "basal foot" is found on the posterior side of the basal body (Fig. 6). At its base the plasma membrane covering the surface of the kinocilium is continous with the plasma membrane of the hair cell top. The hair bundle protrudes into dome shaped cavities on the lower surface of the tectorial membrane with which each hair top is in close contact.

The sensory cell body

The slightly ovoid nucleus of the sensory cell is located at a constant distance from the cell surface. It is thus located in the bottom of the short sensory cells; the size of the infra nuclear region however increases with the length of the cell (Figs. 8–10).

The cytoplasm has the usual organelles, with an abundance of rod shaped

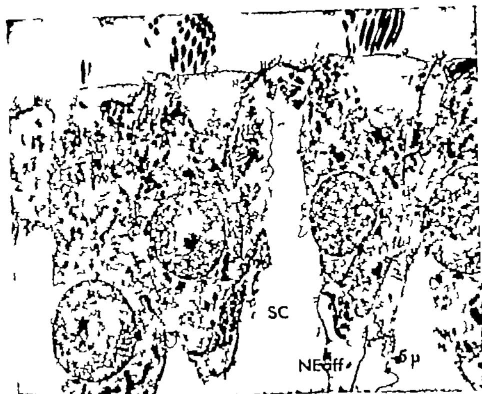


Fig. 8. The hair-cell body exhibit the cell lar organelles normally present with bu dance of mitochondria i the peal and bottom part f the cell. Large lub-shaped afferent adings (NE ff) are present with condensation at the ynaptic contact and globular synaptic body in the hai cell (arrow) Supporting cell (SC) 5000

mitochondria in the apical cytoplasm and the synaptic region. These mitochondria have a diameter of about 0.25 μ and a length of about 1 μ The mitochondrial cristae are transversely arranged

Ribosomes and glycogen granules are scattered throughout the cytoplasm, and lysosome-like inclusions are found in the apical cytoplasm, together with a rich endoplasmic reticulum of the granulated type The Golgi apparatus, with its membranes, vesicles and vacuoles, is also found in this region

The cytoplasmic membrane is smooth, and has a "tight junction where it makes contact with the supporting cells close to the fluid surface This junction is f llowed by an intermediate one and desmosomes as described originally by Farquhar & Palade (1963) and in the inner ear by Lundquist (1965)

The syn ptic region

Both afferent and efferent nerve ending are in contact with the base of the sensory cells. At the synaptic contact between the affe ent nerve ending and the sensory cell a synaptic body is found. This is pherical in shape, and about 0.2 μ diamete and is in close contact with the plasma membrane.



Fig. 9 A sh rt h ir cell with basally loc ted n l nd m ll eff re t n rs di g (NE ff) 0000



Fig. 10. The hexagonal ppearance of the hair cell is pparent in cross-section. 1600.



Fig. 9 A sh rt hair cell with b sally located u l nd sm ll eff rent n ndl g (NE ff) 9000



Fig. 12. A fferent (NE ff) nd efferent (NE eff) nerve-endi g i ynaptic contact with hai cell HC) Not the dense accumulatio f synaptic esicles i the fferent endi g 22,000



Fig. 11 The nerve endings re often l rge and club-shaped. In this fferent nding (NE aff) the synapti contact is recognized by a condensation in the ytoplasm at both the nerve nding and at the side f the hai ell. H re a globular synaptic body (*SB*) is found urrounded by ynaptic vesicles. Hair cell (*HC*) 16,500

The synaptic body is surrounded by a dense accumulation of synaptic vesicles (Fig 11) Similar bodies have been described in the synaptic region of vestibular sensory cells in fishes (Wersall & Flock, 1962) and frogs (Gleisner *et al* 1967) Close to the plasma membrane on both the hair-cell and nerve-ending sides of the synaptic space there is a thin layer of an electron-dense substance (Wersall *et al* 1967)

The afferent nerve endings contains a few scattered vesicles, mitochondria and nerve fibrils.

In the synaptic region of the efferent nerve ending the hair cell is provided with a synaptic cleft this is contributed by a synaptic sac formed by a *membrane bound flat space located close to the synaptic membrane* There is also a condensation of the cytoplasm at the synaptic contact (Fig 12) A synaptic sac of this type was originally described by Engstrom & Wersäll (1955) in the cochlear hair cells of the guinea pig and has since been found in the synaptic area of the efferent endings in all sensory epithelia of the acoustico-lateralis system (Wersäll 1956 Wersäll & Flock 1966 Wersäll *et al* 1967)

The efferent nerve ending is filled with synaptic vesicles and is thus similar in structure to that found in mammals, fishes and amphibia



Fig. 14. The tegmentum asculosum consists of dark cell with a n mber of cytoplasmic projections filled with mitochondria. The l ght cells, the othe hand, interdig tat rith thin, more illiform processes l betwee the other A rich capillary network is seen. 4800

T ctorial membrane

The tectorial membrane is composed of densely packed fibrils with a diameter of about 100 Å, embedded in an amorphous ground substance These fibrils are oriented with a main axis in th anterio-posterior direc tion.

Tegmentum vasculosum

The tegmentum is composed of dark and light cells. The dark cells form the apical part, reaching the surf ce of tegmentum, with a constricted neck and a most irregularly shaped basal part containing the nucleus. This bottom part gi es off long protrusions, which digitate with ones from the light cells. The nucleu of the dark cells, which i slightly irregular in shape, contains fairly dense chromatine network. The cytoplasm is extremely dense, filled with packed mitochondria f various shapes and lengths. The plasma mem bran form multiple folds into the cell to increase the surface area. There is pecial concentration of the rod-shaped mitochondria in these infoldings. The structure f the dark cell in the tegmentum of the cock is very similar to that in the pigeon as described by Dohlman *et al* (19 9)

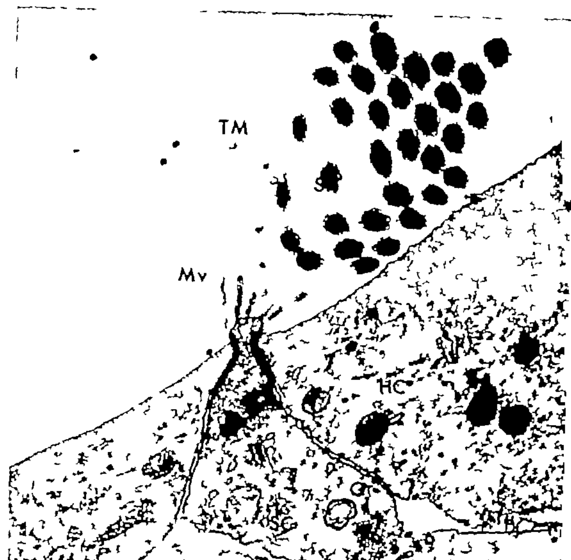


Fig. 13 The secret ry r supporting cell (*SC*) have a t ft of microvilli (*Mv*) at the cell surface. These villi are in clos contact with strands of fibrillar material from the tect rial membrane (*TM*) A centri le (*C*) and granules are f und in th cytoplasm Hair cell (*HC*) with transsect d sensory hairs (*SH*) 20 000

The supporting cells

The cytoplasm of the supporting cells seems to contain a large amount of water The cytoplasmic organelles are scattered throughout the cell with a few mitochondria, endoplasmic reticulum granules and vesicles, most of which seem to be concentrated in the apical portion of the cell The cell surface facing the endolymph bulges slightly above the surface of the neighbouring sensory cells A tuft of microvilla approximately 0.5 μ long and 500 Å in diameter protrudes from the cell surface Each microvillus has a condensed core The microvilli are in close contact with the strands of amorphous and fibrillar material connecting the supporting cells with the lower surface of the tectorial membrane The appearance of secretory granules in the apical portion of the supporting cells suggests that they are concerned in the formation of the matrix of the tectorial membrane

electrodes, Nelson Kiang found inhibition of the spontaneous activity of the nerve fibers when the basilar membrane of the organ of Corti was moved towards the scala tympani, whereas movements in the opposite direction produced an increase in nerve activity. There is convincing evidence that an in-and-out motion of the oval window will result in an anterio-posterior shearing motion between the tectorial membrane and the sensory epithelium of the basilar papilla in birds. This would mean that posterior displacement of the sensory hairs would result in an increase in activity whereas displacement in the opposite direction would produce an inhibitory effect. According to the morphological findings the kinocilium of the hair bundle is always located on the posterior side of the hair bundle. A displacement of the sensory hairs is the adequate stimulus for the sensory cell.

It was originally shown by Löwenstein & Wersäll (1959) and discussed by *Löwenstein et al.* (1964) that a shearing force acting upon the sensory hairs of the vestibular sensory epithelia in the ray increases the nerve activity when the force is directed towards the kinocilium with an increased activity in the innervating nerve fibers and that an inhibition takes place when a force acts in the opposite direction. The present study indicates that the same might be true for the basilar papilla of birds. The actual importance of the kinocilium of the sensory cells for the stimulation of the sensory cells has been discussed elsewhere. It is clear that animals provided with mechano-receptors, where all sensory hairs are structurally similar to kinocilia, must depend on a transducing mechanism in which the mechanical energy is transformed to the sensory cell by the kinocilia. The fact that organ of Corti in the adult mammal has no kinocilia and that some sensory cells even on the basilar papilla have only rudimentary kinocilia, if any, indicates that the kinocilia are not essential to these organs for the transformation of the mechanical energy to the sensory cells. Wersäll (1967) has suggested that the site of the kinocilium and the centriole merely indicates a morphological polarization at the molecular level that is closely linked to the physiological directionsensitive transducing mechanism of the cells.

The appearance of the synaptic apparatus, which is similar in all mechano-receptors of the acousticco-lateralis system, and is composed of a synaptic complex with synaptic membrane, bodies and vesicles, indicates that the synaptic complex is of great significance for the transformation of sensory cell action on the nerve endings and nerve fibers. It is likely that during stimulation of the sensory cell a transmittor substance is released and that the site of this transmittor substance is closely related to the synaptic complex (Vinnikov et al. 1965, Wersäll et al. 1967).

Although it is difficult to draw further conclusions from the present studies to the relation between the peripheral and the central part of the hearing organ in birds for the analysis of the sound, the primitive organization of the basilar papilla, even at the ultrastructural level, clearly supports Schwartzkopff's view (1957, 1958, 1960) that the central analysis must be of greater importance in birds than in mammals.

DISCUSSION

The sensitivity to and frequency discrimination of pure tones in many birds differs slightly from that of mammals (*Heise, 1953* Knecht 1940 Schwartzkopff 1960) Morphological and evolutionary studies of the papilla basilaris in birds indicate, however that their peripheral sense organ of hearing is much less well developed than that of mammals The sensory cells are far less well organized in the papilla basilaris of birds than in the organ of Corti of mammals. Although a difference in length of the cells can be observed there is no structural differentiation that would justify the recognition of two sensory cell types in the basilar papilla. The sensory cells themselves are most similar in structure to the type II sensory cells of the vestibular epithelia in birds and mammals and to the only known cell type in the vestibular sensory areas of fishes. The random distribution of the cell organelles contrasts with the organization of the mitochondria and the fenestrated membranes of the similar rod shaped outer hair cell in the mammalian cochlea

The kinocilium in cochlear hair cells of mammals is found only in the early developmental stage of these cells, and it normally disappears in adult animal (Kikuchi *et al.*, 1965 Kimura, 1966 Wersall & Flock, 1966) The fact that a kinocilium is to be found in the sensory cells of the papilla basilaris, even in the adult animal indicates that these are to be looked upon as more primitive cells than those of the organ of Corti in mammals. These findings further support Löwenstein s view expressed in 1956, according to which the actual linkage between the sensory epithelia and the outer air will determine whether a sensory epithelium will respond to sound or gravitational stimuli The bulky appearance of the basilar membrane and the basilar papilla suggests that the basilar papilla would respond poorly to travelling waves It has been demonstrated by Békésy (1944) however that a mechanical frequency analysis based on the principle of travelling waves in the basilar papilla actually takes place even in the bird. This was in fact suggested by Hasse (1867) a century stage. There is a reason to believe that a vibration of the columella will give rise to a shearing force that affects the sensory cells and the tectorial membrane mainly in anterio-posterior direction. This shearing force would act on the sensory hairs of the hair cells, which in turn regulate the activity in the innervating nerve fibers. Apparently of major significance for the understanding of the function of the organ of hearing is Schwartzkopff's (1958) discovery that the direction of movement of the basilar membrane is colsely related to the formation of the cochlear microphonic recorded from the neighbourhood of the sensory epithelium Only a movement of a basilar membrane towards the oval window will stimulate nerve fiber activity and Schwartzkopff's (1958) finding that a movement in the opposite direction inhibits the nerve activity would seem to be of particular importance This is true also of the basilar membrane movement in the cochlea of the cat where by analysis of a great number of nerve fibers of the acoustic nerve studied with micro

electrodes, Nelson Kiang found inhibition of the spontaneous activity of the nerve fibers when the basilar membrane of the organ of Corti was moved towards the scala tympani, whereas movements in the opposite direction produced an increase in nerve activity. There is convincing evidence that an in-and-out motion of the oval window will result in an anterio-posterior shearing motion between the tectorial membrane and the sensory epithelium of the basilar papilla in birds. This would mean that posterior displacement of the sensory hairs would result in an increase in activity whereas displacement in the opposite direction would produce an inhibitory effect. According to the morphological findings the kinocilium of the hair bundle is always located on the posterior side of the hair bundle. A displacement of the sensory hairs is the adequate stimulus for the sensory cell.

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ACTIVITY OF ACETYLCHOLINESTERASE ON THE ENDOLYMPHATIC SURFACE OF OUTER HAIR CELLS

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By means of the Karnovsky's modification of Koelle's thiocholine method for acetylcholinesterase (AChE) it has been demonstrated electronmicroscopically that AChE is located on the endolymphatic surface of outer hair cells and not on the supporting cells in the organ of Corti of the guinea pig cochlea. The tissue from the middle part of the basal turn was used in all experiments. The identification of AChE was confirmed by use of appropriate concentration of selective inhibitors of cholinesterase. From the inhibitory effect of fixatives on AChE activity it was obvious that the activity on the surface of outer hair cells was lower than the activity on the large nerve endings at the bottom of the hair cells.

The surface of the sensory hair cells of the organ of Corti has been of great interest because of its potential as a functional interphase between the endolymph and the hair cells. Tasaki *et al* (1952) suggested that the upper part of the surface membrane of the hair cells plays an important role in generating the cochlear microphonic Vinnikov & Titova (1964) demonstrated with light microscopy acetylcholinesterase activity in the hairs of the inner and outer hair cells. The authors suggested that the surface of the hair cells may be a special type of postsynaptic membrane. Recently histochemical techniques have been combined with electronmicroscopy in order to localize enzyme activity in specific structures of individual cells The present study has employed the combined technique to investigate acetylcholinesterase activity on the endolymphatic surface of the outer hair cells of the organ of Corti

METHOD

The temporal bones of normal guinea pigs were obtained immediately after sacrifice and placed in a cold fixative solution Two fixatives were used, (1) 2.5% glutaraldehyde buffered with 0.1 M phosphate (pH 7.2) and (2) 4% formaldehyde buffered with 0.1 M of the same buffer including 4.0% sucrose During fixation the bony capsule was removed and the tissue

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of each turn of the cochlea was separated. The duration of fixation was determined after studying the effects of fixation time on the enzyme activity Fixation time varied up to 4 hours for glutaraldehyde and up to 27 hours for formaldehyde. A maximum fixation time of 25 minutes was selected for glutaraldehyde and 4 hours for formaldehyde.

The tissues were incubated in Karnovsky's media (1964) for acetylcholinesterase activity This consisted of 5 0 ml of sodium citrate (1 M) 1 0 ml of copper sulfate (30 mM) 1 0 ml of water 1 0 ml of potassium ferricyanide (5 mM) 5 g of sucrose and 6.5 ml of acetylthiocholine as substrate. Incubation was carried out at 4 C for 15 minutes.

A second set of experiments was designed to show the effect of specific inhibitors on acetylcholinesterase activity The tissue was first incubated in the phosphate buffer solution for 25 minutes to which eserine 1×10^{-4} M or BW 62C47 3×10^{-4} M had been added and then transferred to the complete media with the substrate and the same amount of inhibitors (BW 62C47 was supplied by Burroughs Wellcome Co) Controls were run by incubating the tissue in the media without the substrate acetylthiocholine After incubation the tissue was washed briefly in 0.2 M sucrose buffered solution and post fixed f r 1 / hours in 1% OsO4 buffered with veronal acetate The tissue was dehydrated in a graded series of ethanol solutions and embedded in Epon. The tissue was sectioned on an L.K.B microtome and studied with a Siemens Elmiskop 1 Most of the sections were stained slightly with uranyl acetate and lead citrate to enhance contrast

RESULTS

In all of the experiments only the out r hair cells, supporting cells and Hensen s cells from the middle of th basal turn of the cochlea were ex amined. When the tissue was incubated in the complete medium containing acetylthiocholine as ubstrate, small electron opaque granular reaction product ppeared on the plasma membrane surface of the outer hair cells. Neither the supporting cells, nor Hensen cells showed these deposits on their surface membrane There were no deposits within the cytoplasm of either the out hair cells, r the upporting cells. (Figs. 1 2 *a* *b* *c* 3 *a*, 4 *a*) The reaction product w re also present on the large nerve endings at the base of th hair cells. These deposit on the nerve endings had been demonstrated bef re (Ka ko & Daly 1967 in press)

In order t dem nstrate that the granular products were the result of acetylcholinesterase (AChE) activity specimens f tissue w re incubated in media without th substrat In these xperiments granular products were not seen on the surface of the hair cells, however finely divided percipitates w re seen (Figs. 3 *b* 4 *b*)

N xt perim n f tissue were incubated in complete media containing substrat t which had been added the inhibitors of cholinesterase eserine 1 10 M nd BW 62C47 3 10^{-4} M (Fig. 4 *d*) In these experiments granu-

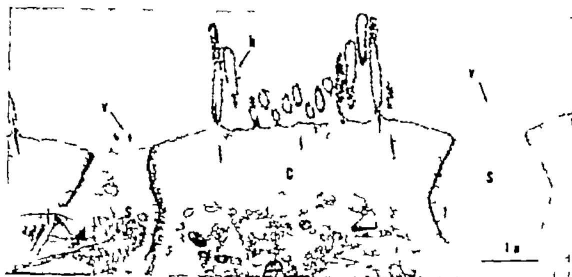


Fig 1 Tangential section through the t p of out r hair cells of 1st row End lymphatic surface of hai cell including th pl ma membrane of hair show the reaction products. No reaction product on the supporting cells. Prefixati n with formaldehyde 45 minutes. Incubation time 10 minutes *S, supporting cell (reticular membrane) C, cuticular l te* of hair c ll *h* hair (stereocilia) on hai cells *v* microvilli on supporting lls.

lar products did not appear on the surface of the hair cells. It was concluded that the granular products were the result of specific cholinesterase activity The fine precipitates appeared when the substrate was absent from the media and also in experiments in which the cholinesterase inhibitors were added. It was concluded that the fine precipitates were not due to the AChE activity but due to some other reduction reaction

After prolonged fixation with glutaraldehyde or formaldehyde both the reaction products and the fine precipitates either diminished or did not appear at all The reaction was completely stopped by 2.5% glutaraldehyde after 60 minutes, and almost completely inhibited by formaldehyde after 27 hours. Although the preservation of the tissue fixed in glutaraldehyde was fairly good the tissue showed the non-enzymatic precipitation of granular material within the cells as reported by Schlaepfer & Torack (1966) (Fig 5 *a*)

DISCUSSION

In this study it was demonstrated that the reaction products on the surface of the outer hair cells incubated in the complete incubation medium disappeared from the tissue which was incubated in medium lacking substrate The use of eserine 1×10^{-4} *M* and BW62C47^{-4} *M* as inhibitors of cholinesterase produced complete inhibition of the reaction products on the surface of the outer hair cells as well as on the large nerve endings at the bottom of the hair cells The concentration of inhibitors used here are generally acceptable in histochemical work (Pearse 1961) with electron microscope (Barrnett 1962 Karnovsky & Hug, 1963 Karnovsky 1964

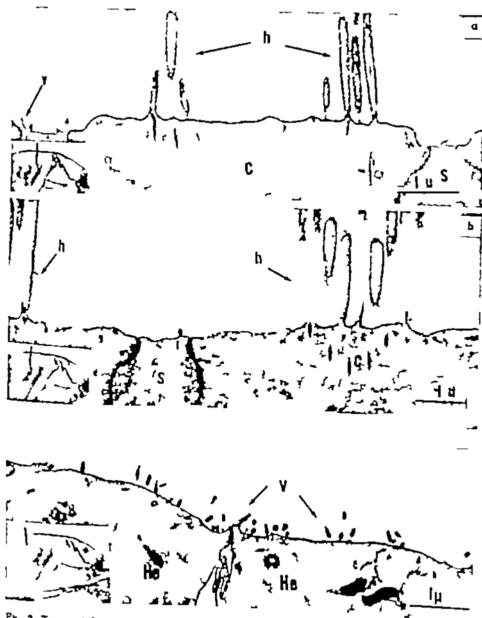


Fig. 2. T ngential section through the top of ( ) outer hai cell of 1st row (*b*) uter hair cell of 2nd row (H nsen cells. Reaction product re excl sively located on the urface of the hair cell nd not on the supporting cells. The urfac of Hensen cells do not show the reacti products. Prefixation w th formaldehyde 45 minutes. Incuba- tie time 10 min tes.

Bloom & Barrnett, 1966 Brizin *et al* 1966 Schlaepfer & Torack, 1966 No- ik. *t al* 1966) Following the classification of esterases given by Pearse the nzyme i ither cholinesterase or an aliesterase, both of which are sensit e to thi concentration of eserine.



Fig. 3 (*a*) High magnification of reaction product on the surface of hair cell incubated in complete incubation medium show that the small granular products are continuously located on the plasma membrane of hair cells (mostly located on the plasma membrane facing inside of hair cell). (*b*) High magnification of precipitates on the surface of hair cells incubated in medium lacking substrate. Indefinite granular precipitates with low density are located on the surface of hair cells and not on the supporting cells. Comparing the precipitates with the reaction products of Fig. 3*a* it is obvious that there are differences between them in size and density. Prefixation with formaldehyde 3 hours. Incubation time 15 minutes.

Furthermore the specificity of acetylthiocholine for cholinesterase (Augustinson 1963, Koelle 1963) and inhibition by specific cholinesterase inhibitors indicates that the reaction products on the endolymphatic surface of the outer hair cells are due to the specific cholinesterase. The reaction products did not occur in the supporting cells or Hensen's cells.

It is generally admitted that the first step in the transformation of mechanical acoustic energy into electrical energy occurs at the level of the hair cells. Although it has been established electrophysiologically that the cochlear microphonic arises in the region of the upper end of the hair cells (Tasaki *et al* 1952) the exact place of its origin is not yet known.

In their electrophysiological experiments Tanaka & Katzuki (1966) and Katzuki *et al* (1965) found pharmacological activity of acetylcholine and d-tubocurarine in the vicinity of the hair cells. The findings of specific cholinesterase activity on the surface of the hair cells suggests that a cholinergic mechanism is present on the endolymphatic surface of the hair cells.


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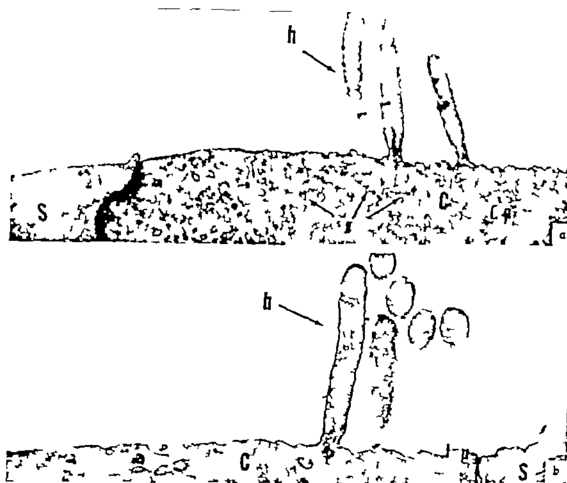


FIG. 5 (*a*) The tissue prefixed with glutarald hyde for 90 mi utes and incubated in complete in ubation medium. R acti n products a d fine precipitates on the urface f hair cell were not seen. N n-specifi granular prod cts (*g*) appeared in the tissue. (*b*) The tissue incubated in ph sphate buffer—no reacti n products.

When the tissue was incubated in the medium lacking the substrate there still remained fine precipitates on the endolymphatic surface of the outer hair cells, also on the hairs themselves The formation of the fine precipitates was not affected by the cholinesterase inhibitors It is possible that these precipitates are the result of the reduction of ferricyanide to ferrocyanide, for instance by SH group in the surface of hair cells. However this will require further investigation

FIG. 4 (*a*) The ti u incubated in complet ncubati n medium. (*b*) Co trol prepara ti n. The tissu cubat d in medi m la king ubstrate ( ) I hibiti n perim nt The tissu tre ted by inh bit r Eseri 10^{-4} *M* with complet bati n medium (*d*) Inhibi tion perim nt. The ti u treated by i hibitor BW 62C47 5 10^{-4} *M* with compl t incuba tion medium. In Figs. 4 *b–d* small tiny precipit t re located n th rf ce f hair cell including the hair nd not n the supp rt cells. Th localizati n f these p ecipitates is n f the re cti n prod ct of Fig. 4 *a*, b t th d n ity and si of th se precipitates re less and smalle than f m th re ti n product Prefixati n w th f rm ld hyde 2 h urs. Incubation tim 25 minutes.

Many investigators have reported that prefixation is necessary to maintain not only optimal structural integrity but also to obtain a sharp histochemical localization of cholinesterase (Couteaux, 1958 Lewis & Shute, 1966 Shimizu & Ishii, 1966 Shute & Lewis, 1966 Robinson & Bell, 1967 Eranko *et al* 1967) However when the tissue was fixed in the glutaraldehyde for more than 40 minutes the reaction products on the surface of hair cells did not appear Koelle (1963) has reported on the inhibitory effects of glutaraldehyde on the activity of cholinesterase. On the other hand, the reaction products were still located on the large nerve endings at the bottom of the hair cells even after long fixation with glutaraldehyde (Kaneko & Daly 1967) This difference between acti ity on the surface of the hair cells and in the nerve endings at the bottom of the hair cells was constant. We are n t able to explain this difference other than to suggest there may be a difference in the strength of the reaction of these sites. It maybe that the activity of specific cholinesterase on the surface of the outer hair cells is weak and can be easily inhibited by glutaraldehyde.

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The authors wish to ackn wledge the constructi e criticism and helpf l advice g en by Dr K. Cheng. The valuable technical assistance f electronmicroscopic preparation of Mrs G. Richardson, and the histochemical preparations of Mr R. Gore are also gratefully acknowledged.

ZUSAMMENFASSUNG

Mit Koelle's Thiocholinmethode zum Nachweis on Acetylcholinesterase (AChE) modifiziert nach Karnovsky wurd lektronenmikroskopisch gezeigt, dass im Corti schen Organ des Meerschwein hen AChE ausschliesslich auf der endolymphatischen Oberflache der ausseren Haarzellen lokalisiert ist, nicht aber auf der Oberflach der Stutzzellen Unsere Untersuchungen beziehen sich auf Gewebe om mittleren Teil de Basalwindung der Cochlea. Der Na hweis von AChE *wurde bestatigt durch Verwendung entsprechender Konzentration* n on Cholinesterasehemmern Au der Hemmungswirkung von Fixierungsmitteln auf die AChE-Akti i t der Oberflache wurde deutlich, dass di Aktiv i t auf der Oberflache der usseren Haarz llen geringer ist als di Aktivitat an den grossen Nervendigungen m unteren Teil der Haarzellen.

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THE ABSENCE OF EFFECTS OF ATARAX ON AUDITORY FATIGUE

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In a partial replication of a previous experiment by Wojnarowska, 12 normal-hearing adults between th ages of 18 and 26 were given oral 50- or 10-mg doses of atarax o either of two placebos prior to an 8-min exposure to a 1500-Hz tone at 105 dB SPL. Three control and four experimental sessions were conducted. Comparison of pre- and post exposure thresholds for these indicated that neither the drugs nor the placebos had a significant effect on either the mount or duration of auditory fatigue.

There has been little systematic investigation of the effects of pharmacological agents on auditory fatigue, particularly as it occurs in humans (as opposed to guinea pigs) The few studies concerned with drug effects in humans vary considerably in method, drug type and dosage, and in frequency intensity and duration of fatigue-producing stimuli. Thus results are not comparable, and iewed as a body they provide no conclusive evidence as to the nature of the relation between the administration of pharmacological agents and the extent and duration of temporary shifts of hearing threshold (TTS) indicative of auditory fatigue.

For example some attention has been focused on the effects of vitamin A. Willemse (1952) exposed five normal hearing subjects to a 2000-Hz tone at 120 dB for 8 min before and after each received daily doses of 300 000 units f vitamin A He found that fou of the five demonstrated a slight reduction in the amount of TTS following the vitamin therapy However in a controlled study Ward & Glorig (1960) found that daily doses of 100,000 units of itamin A gi en over the course of a week, had no effect on TTS. Their subjects w re 15 normal hearing males who were exposed for 1 hr to octave-band noise of 2400–4800 Hz t 100 dB SPL. These authors pointed out that vitamin A i a drug whose significanc for sensory processes is normally reflected by its absence and there is no reason to suppose that individuals without A vitaminosis will show impro ement in sensory functioning as a result of additional doses of itamin A.

H rm ne effect have been examined by Matsui *et al* (1963) They indicate that prior t a 1-hr exposure to white noise, the administration of either estriol or estradiol produced a significant reduction in the duration of TTS in 11 men but that the administration of testosterone had no effect

on either the amount or duration of TTS in seven women. However these authors did not clearly specify the nature of their control groups or the intensity of the fatigue-producing stimulus they used They reported the latter only as being "90–95 phons"

Other drugs examined for a possible influence on auditory fatigue have included quinine, phenamine, and 5 phenyl 2 imin-4 oxo-oxazolidine. Womack (1961) administered three daily doses of 300 mg of quinine sulfate to seven men He found no significant differences between this group and a control group which had received placebos, in TTS at 4000 Hz following a 7 min exposure to white noise at 117 dB SPL. Chernyak (1958) reports, though without details, that the administration of phenamine 1 hr before noise exposure allegedly produced a significant reduction in TTS in most subjects tested Similar claims (and similarly unaccompanied by detailed data) are made for chondroitin sulphate, vitamin B_1 and pantothenic acid (Kubo 1963)

One of the few drugs which has been alleged to produce an increase in auditory fatigue is 5 phenyl 2 imin-4-oxo-oxazolidine Miraglia & Amorelli (1962) have reported the results of several experiments in which a potentiation of fatigue was noted following the administration of this drug Unfortunately only a brief summary of their work has been available in translation, and this gives no indication of the drug dosages used the duration of its administration or the nature of the exposure stimuli whose effects it was found to increase.

The most recent study is that of Wojnarowska (1966) who examined the effects of several stimulants and tranquilizers on auditory fatigue in 20 adults Stimulants tested were strychnine, caffeine, and lobeline tranquilizers tested were luminal atarax, and meprobamate Of these, two tranquilizers, atarax and meprobamate, and one stimulant, caffeine, were claimed to have produced a significant reduction in both the amount and duration of fatigue She reported that following an 8-min exposure to a 1500-Hz tone, mean TTS (at an unspecifed frequency) was normally 20 dB requiring 8.2 min to recover while following administration of atarax mean TTS was 11 dB requiring 3 6 min to recover

Because of the striking nature of these results (both stimulants and tranquilizers showing the same effect) and because of certain methodological problems felt to be inherent in Wojnarowska s study it was decided to conduct a partial replication of her experiment, using atarax, the tranquillizer she found to yield the most significant results The following is a report of experimentation designed to test the effects of this drug on auditory fatigue patterns in 12 normal hearing adults

METHOD

Although the experiment was intended to be a replication of Wojnarowska s work involving atarax several modifications of her method were

affected in order to achieve a greater degree of experimental control These concerned the intensity of the exposure stimulus, the procedure for threshold-testing before and after exposure, the drug dosage, and the manner of its administration.

Wojnarowska s exposure stimulus consisted of a 1500-Hz tone, presented for 8-min at 75 dB above a predetermined threshold (Wojnarowska, 1966) In preliminary tests for the current investigation, it was found that, for a 1500-Hz tone, the minimum intensity required to produce a signifi cant (15 dB or more) and consistent level of fatigue in normal-hearing subjects was 105 dB SPL (98.5 dB HL ISO 1964) Consequently this was the intensity selected for use.

Wojnarowska s threshold testing was conducted with a 5-dB discrete step audiometer In order to obtain more finely-grained measurements and to reduce the probability of experimenter error in testing, it was decided to employ Békésy audiometry This allows for continuous and automatic recording of a subject s threshold and its variation in estimation to 1-dB accuracy In addition, it was decided to measure post-exposure thresholds continuously for 8 min, a period equal to that of the exposure duration. It was felt that this would yield a more complete pattern of the progress of recovery than would measuring thresholds at 2 min intervals as Wojnarowska had done.

The dosage of tarax received by her subjects was 10 mg, administered orally in a single-blind procedure (Wojnarowska, 1966) According to the medical director of J B. Roerig & Sons, the U S. manufacturer of this drug, atarax is usually dispensed in 50-mg tablets, and the normal, daily adult dosage is four of these or 200 mg (Fowler 1968) Because of this fact, it was decided to examine the effects of both 50-mg and 10-mg doses of atarax in the current investigation, even though Wojnarowska used only 10-mg doses.

Finally to avoid xperimenter bias, it was f lt necessary to use a double-blind procedure in the test administration.

The subjects were five m n and seven women. Their ages ranged from 18 to 26 years with a mean of 23 years. Ten were college students, and two were Hearing Research Laborat ry personnel. The results of Békésy audiometry conducted within a week pri r to the experiment, indicated that all had normal hearing, as defined by thresholds no greater than 15 dB HL (re ISO 1964 tandard) for frequencies of 1000 1500, 2000 and 4000 Hz. In th course of this testing, all demonstrated the ability to respond to a Békésy udiometry procedure with variation of 7 dB or less in threshold rang Th ubject reported themselves to be without colds, ear infections, or othe ear probl ms. None was taking any medication at the time of the xperiment.

Pure tones f testing were generated by a Hewlett Packard oscillator Model 201CR, nd shaped with a Grason Stadle 829C electronic switch to yield 250-msec tone pulses with 25-msec rise- and decay-times and a 250-

on either the amount or duration of TTS in seven women. However these authors did not clearly specify the nature of their control groups or the intensity of the fatigue producing stimulus they used. They reported the latter only as being "90–95 phons."

Other drugs examined for a possible influence on auditory fatigue have included quinine, phenamine, and 5-phenyl 2 imin-4 oxo-oxazolidine. Womack (1961) administered three daily doses of 300 mg of quinine sulfate to seven men. He found no significant differences between this group and a control group which had received placebos, in TTS at 4000 Hz following a 7 min exposure to white noise at 117 dB SPL. Chernyak (1958) reports, though without details, that the administration of phenamine 1 hr before noise exposure allegedly produced a significant reduction in TTS in most subjects tested. Similar claims (and similarly unaccompanied by detailed data) are made for chondroitin sulphate, vitamin B_1 and pantothenic acid (Kubo, 1963).

One of the few drugs which has been alleged to produce an increase in auditory fatigue is 5-phenyl 2 imin-4-oxo-oxazolidine. Miraglia & Amorelli (1962) have reported the results of several experiments in which a potentiation of fatigue was noted following the administration of this drug. Unfortunately only a brief summary of their work has been available in translation, and this gives no indication of the drug dosages used, the duration of its administration, or the nature of the exposure stimuli whose effects it was found to increase.

The most recent study is that of Wojnarowska (1966) who examined the effects of several stimulants and tranquilizers on auditory fatigue in 20 adults. Stimulants tested were strychnine, caffeine, and lobeline; tranquilizers tested were luminal, atarax, and meprobamate. Of these, two tranquilizers, atarax and meprobamate, and one stimulant, caffeine, were claimed to have produced a significant reduction in both the amount *and duration* of fatigue. She reported that, following an 8-min exposure to a 1500-Hz tone, mean TTS (at an unspecifed frequency) was normally 20 dB requiring 8.2 min to recover, while following administration of atarax mean TTS was 11 dB requiring 3.6 min to recover.

Because of the striking nature of these results (both stimulants and tranquillizers showing the same effect) and because of certain methodological problems felt to be inherent in Wojnarowska's study, it was decided to conduct a partial replication of her experiment, using atarax, the tranquilizer she found to yield the most significant results. The following is a report of experimentation designed to test the effects of this drug on auditory fatigue patterns in 12 normal hearing adults.

METHOD

Although the experiment was intended to be a replication of Wojnarowska's work involving atarax, several modifications of her method were

sented in the following order 2000 1500, and 1000 Hz to the left ear 1000 1500, and 2000 Hz to the right ear and 4000 Hz to the left ear With the exception of the first presentation to the left ear of 2000 Hz, which was of 40 sec duration, each frequency was tested for 20 sec. The above order was repeated until a total of 8 min had elapsed. Thus, following exposure, four measurements were obtained for 2000 Hz in the left ear and three for each of the other frequencies in either ear

Thirty minutes before each of the four experimental sessions, the subjects were given oral doses of either the drugs or the placebos. Because the 50-mg atarax was in capsule and the 10-mg in liquid (10 mg/dram) form, two placebos, one in each form, were used. The 12 subjects were divided into four groups, and each group of three received the drugs and placebos in a different order in alternating liquid and capsule forms. All doses were administered by the experimenter under the supervision of Dr Arndt J Duvall, III. The subjects received them, followed by 4 oz. of water at least an hour after having eaten As mentioned previously a double-blind procedure was employed, so neither the experimenter nor the subjects knew whether they w re being given drug or placebo Prior to each administration, the subject were told the following

> This is one of the 4 sessions in which you are being given either a mild tranquilizer or a placebo. Both are in liquid and capsule forms. For today you will swallow this capsule (liquid) After 30 minutes, your hearing will be tested before and after you listen to the loud sound. During the 30-minute waiting period, you are asked n t to smoke and not to eat or drink anything. If you feel drowsy at ny time foll wing the experimental session, you are cautioned not to drive and not to operate any dangerous machinery

RESULTS

Pre- and post-exposure threshold values were determined by visual estimation f the mid point f the Békésy tracings for each frequency To obtain an indication of the reliability of these measurements, ne session s tracing for ea h ubject were measured by the experimenter (NW) on two separate occasions, usually a day part. Comparison of midpoint values a signed a threshold indicated maximum variation of only 1 dB in either direction for any frequency the mean difference disregarding sign, was 0 12 dB

The mean f pre-exposure thresholds for all frequencies in each ear were det rmined. nd their comparison f r all sessions showed maximum variability t be 2 dB between ny two session with a net shift of less than 1dB Thu neith r the drug nor the placebos had any significant effect n threshold of audibility

M an sh fts, computed f r all frequencies in each ear revealed between-sessi n diff rence of less than 2 dB. Within thi range immediate post

msec silent period. A Grason Stadler E3262A recording attenuator changed the level of the test signal at the rate of 4 dB per sec. The continuous exposure tone was generated by a second Hewlett Packard 201CR oscillator Voltages across the earphones were calibrated for all tones with a Ballantine 300G vacuum tube voltmeter at the beginning of each day The tones were presented through PDR8 earphones in MX-41/AR cushions in an Industrial Acoustics sound treated room model 1202A

PROCEDURE

Seven sessions, three control and four experimental, were conducted within a 2 wk period for each subject. Two control sessions preceded and one followed those involving the drugs and placebos. Prior to the first session, the subjects were told the following

> You are being asked to participate in an experiment designed to test the effects of 2 different, but harmless drugs on hearing Altogether there will be 7 experimental sessions. During each, your hearing will be tested then you will be asked to listen to a loud sound for a few minutes, and then your hearing will be tested again Before 4 of the sessions, you will be given either a mild tranquilizer or a placebo both will be in liquid and capsule forms. On 2 occasions you will receive the drug, and on 2 occasions, the placebo These 4 sessions will take about an hour each the other 3 sessions will take about a half hour each. Are there any questions?

For each session, the procedure for threshold testing and exposure was the same. The subject was seated in the sound treated room and was given standardized instructions which stressed (1) pressing the voting button just as soon as a single beep was heard and releasing it as soon as one was missed, (2) adjusting headphones for snug fit, and (3) being as quiet as possible while listening, e.g by breathing through the mouth and by holding the cord to the earphones to prevent its scraping against the clothing After making certain that the earphones were seated properly and that the subject was holding their cord as directed, testing was begun.

Thresholds were obtained first for the left ear (the ear to be exposed subsequently) and then the right. Frequencies tested were 1000 1500 and 4000 Hz in that order Each was presented for 30 sec Following the tests for the right ear the threshold in the left ear for 2000 Hz was again determined This was to provide for immediate pre and post exposure comparison since this was the first threshold to be measured following exposure, and this threshold was the one which could be expected to show the greatest degree of shift Then the exposure was begun a 1500 Hz tone at 105 dB SPL was presented, through the earphone to the left ear for 8 min Fifteen seconds before the end of this period, the subject was told to get ready' to respond to threshold testing

Immediately after exposure testing was resumed Frequencies were pre-

and 4000 Hz for the drug, placebo, and control sessions. For the control session data, the function is the combined mean of the second (before-exposure) and the third (after-exposure) sessions without medication. These patterns are plotted logarithmically in time in order to yield linear functions. Their examination reveals the insignificance of the differences in both TTS amount and duration following the administration of the drugs and placebos.

DISCUSSION

These results indicate that atarax, in either 50-mg or 10-mg doses, has no effect in normal hearing listeners on the amount or duration of auditory fatigue produced by an 8-min exposure to a 1500-Hz tone at 105 dB SPL. This is in direct opposition to the striking effects in fatigue-reduction reported by Wojnarowska (1966) with 10-mg doses of atarax, followed by exposure to a similar stimulus at a presumably lower level of intensity. It is possible that atarax if administered in larger or more prolonged doses and perhaps prior to a more intense or prolonged exposure than was used in the present experiment could effect a reduction in auditory fatigue as significant as that reported by Wojnarowska, but it hardly seems likely.

Nevertheless, it may be important that further research efforts be directed toward the examination of the influence of drugs such as atarax on patterns of TTS recovery. This is especially true for drugs which could possibly reduce fatigue effects. Since repeated episodes of fatigue commonly occurring in industrial workers exposed to high levels of noise in their jobs, can lead to permanent hearing loss, pharmaceuticals which could be found to produce a reduction in fatigue might have practical significance for retarding the development of such loss.

ZUSAMMENFASSUNG

Im Gegensatz zu Ergebnissen von Wojnarowska im Jahre 1966 vorgelegt, wurde keine Veränderung in der Hörermüdung, nach einer 8-Min Aussetzung zu einem 105-dB-SPL 1500-Hz Ton gemessen, durch entweder 10- oder 50-mg Portionen Atarax in einem doppelg[illegible] Verfahren produziert.

ACKNOWLEDGMENT

The cooperation and assistance of J. B. Roerig & Sons, Division of Charles Pfizer & Son Pharmaceuticals and of its medical director J. Ralph Fowler M.D. are gratefully acknowledged. This research was supported by grant NB04403 from the U.S. Public Health Service Dept. of Health, Education and Welfare.

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TABLE 1 *Mean initial TTS in dB in the exposed (left) ear at 2000 and 4000 Hz.*

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50-mg atarax	13.6	7.1
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50-mg placebo	14.5	7.2
10-mg placebo	14.9	7.3

[a] Mean of the second control ses ion before the four drug placebo sessions and the control session after the four

exposure thresholds, particularly at 2000 and 4000 Hz in the exposed ear (see Table 1 for mean shifts) did tend to be slightly reduced for both of the drug sessions however differences of this magnitude do not begin to approach statistical significance Thus, the amount of TTS was significantly affected by neither the drugs nor the placebos.

Duration of TTS was also not found to be significantly affected Between session differences in mean final post-exposure thresholds amounted again to less than 2 dB Here, the tendency toward higher values was noted for the drug sessions than for the control sessions, particularly for the exposed ear at 2000 and 4000 Hz. Fig 1 illustrates TTS recovery patterns at 2000

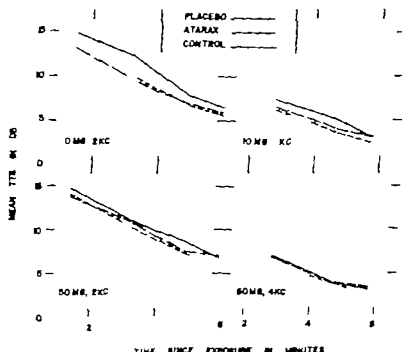


FIG. 1 Course of reco ery for 8 min afte exposure [ ] drug, placebo, nd co trol sessi ns. Drug dosag and test frequ ncy are indicated fo each panel Left ars [ ] 12 ubjects.

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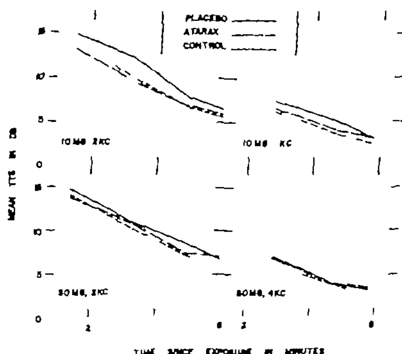


FIG. 1 Course of recovery for 8 min afte xposure, in drug, pl cebo, and control sessions. Drug dosage and test frequ ncy are indicated fo e ch panel Left ears f 12 subjects.

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RHYTHMICAL CEREBELLAR RESPONSES TO LABYRINTHINE STIMULATION

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The electrical activity of the cerebellar cortex has been recorded with microelectrodes simultaneously with the action potentials of a few fibers of an oculomotor nerve during eye nystagmus brought about by thermic stimulation of the labyrinth in curarized guinea pigs. Tonic and rhythmical responses to the labyrinthine stimulation were recorded from uvula, flocculus and parafloccuhus. Rhythmical cerebellar responses in 81% of the cases exhibited the same frequency as that of the eye nystagmus. However in the great majority of cases the rhythmical cerebellar outbursts were not perfectly synchronous with the quick or slow phases of the eye nystagmus. In the remaining 19% of cases the frequency of the rhythmical cerebellar responses was different from that of the eye nystagmus. The conclusion may be reached that the cerebellum although not essential for the appearance of the eye nystagmus is the seat of rhythmical activity during eye nystagmus. Thus the cerebellum utilizes messages from the vestibular apparatus in order to influence and regulate the correct performance of the extra-ocular movements just as it acts on the limb movements.

The relationship of the cerebellum to ocular movements were analyzed from different points of view. Several investigations were devoted to the study of cerebellar influence on eye movements elicited by labyrinthine stimulation and especially on labyrinthine eye nystagmus (Dow & Manni, 1964 Dow & Moruzzi 1958 Manni, 1966) Magnus (1914) and De Kleijn & Magnus (1920) stated that the cerebellum is not necessary for the appearance of all vestibular reflexes and particularly of the eye nystagmus. However further investigations showed that cerebellar asymmetrical lesions and cerebellar stimulations can modify the vestibular reflexes (Aschan *et al* 1963 Bauer & Leidler 1912 Chambers, 1947 Chambers & Sprague 1955 Cohen *et al* 1965 Di Giorgio, 1950 Dow 1938 Fernández & Fredrickson, 1963 Ferraro & Barrera, 1936 Grant *et al* 1963 Hahn 1950 Hare *et al* 1936, 1937 Hoshino, 1921 Koella, 1955 Lorente de Nó, 1931 Le Gros Clark, 1938 Lowe 1946 Magoun *et al* 1935 Manni, 1950 Miller & Laughton, 1928 Moruzzi, 1948 Rasmussen, 1933 Schoolman & Delgado, 1958 Simonelli & Di Giorgio, 1931 Spiegel & Scala, 1941 1942 Whiteside & Snider 1953) The cerebellum receives direct and indirect vestibular

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nystagmus, which could have been recorded if the animal had not been curarized.

The recorded sites in the cerebellum were marked by electrolysis. At the end of the experiments the animals were killed with a large dose of Nembutal. The cerebellum was removed, fixed in alcohol, embedded in paraffin, serially cut and stained with the Nissl's method.

RESULTS

Eye nystagmus elicited by thermic stimulation of the labyrinth

Before curarizing the animals, at the beginning of the experiments, the ocular effects of the thermic stimulation of the labyrinth were controlled. The cold (10 C) stimulation of a labyrinth provoked an ipsiversive slow deviation followed by eye nystagmus with the quick phase towards the contralateral side. The warm (40–45 C) irrigation of the labyrinth induced an eye nystagmus with the quick phase directed towards the stimulated side. All such results are altogether in agreement with those obtained by other investigators (Camis, 1928).

Electrical activity of the cerebellum during thermic stimulation of the labyrinth

The results of the present report are based on the analysis of the records obtained in 23 guinea pigs with 24 histologically checked localizations of the recording microelectrode tip in the cerebellar cortex. The tip of the microelectrode was located in the region of the Purkinje cells. Fourteen out of the 24 localizations were in the uvula (Larsell's lobule IX), seven in the paraflocculus (H. VIII *b* H.IX) and three in the flocculus (H.X) (Fig. 1). The thermic stimulation of the labyrinth induced in all the records modi

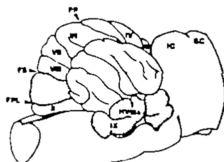


Fig. 1. Lateral view of the cerebellum of the guinea pig. *FP* fissura prima, *FS*, fissura secunda, *FPL*, fissura postero-lateralis. *IC*, *SC*, inferior and superior colliculi. The roman figures indicate the cerebellar folia according to the classification by Larsell. The dotted areas show the cerebellar lamellae (IX, Uvula; H.X, Flocculus; H. VIII *b* Paraflocculus dorsalis, and H. IX, Paraflocculus ventralis) from which the rhythmical responses to the labyrinthine stimulation were recorded.

afferents, which end particularly in the flocculo-nodular lobe, lingula, uvula, paraflocculus and fastigial nuclei (Brodal *et al* 1962 Dow & Moruzzi, 1958 Jansen & Brodal, 1954) On the other hand cerebellar fibers impinge upon brain stem structures—vestibular nuclei, reticular formation, oculomotor nuclei—which are involved in the ocular labyrinthine reflexes (Azzena & Giretti, 1967 Azzena *et al* 1967 Brodal *et al.*, 1962 Carpenter & Strominger 1964 Chatelier & Leitner 1962 Duensing & Schaefer 1957 Dumont Tye & Dell 1961 Gernandt 1964) Electrophysiological studies have confirmed and extended such anatomical findings. Various stimulations of the labyrinth or of the vestibular nerve induced modifications of the cerebellar electrical activity which were particularly evident in the vestibular areas of the cerebellum (Andersson & Gernandt, 1954 Ardumi & Pompeiano, 1957 Camis, 1919 Chang & Kostyuk, 1960 Dow 1939 Gualtierotti & Passerini, 1958 1959 Price & Spiegel 1937 Riva Sanseverino & Urbano 1965 Weber & Steiner 1965)

The present paper was devoted to analyzing the changes in electrical activity of the cerebellum during vestibular eye nystagmus. It shows that under particular conditions the cerebellum exhibits a rhythmical activity in relationship to vestibular eye nystagmus

METHOD

The present experiments were carried out on 52 guinea pigs weighing 400–500 g Under ether anesthesia the animals were tracheotomized, cannulated and put in a stereotaxic apparatus. Following an appropriate craniotomy the cerebellum was exposed and protected by warm mineral oil The frontal poles were also exposed and ablated by suction in order to visualize the oculomotor nerve before it entered the orbit Both epitympanic recessi were opened and cannulated in order to stimulate the labyrinth by means of warm or cold water irrigations The ether anesthesia was stopped and all the operatory wounds were infiltrated by procaine Then the animals were immobilized by d tubocurarine (1 mg i p) and the artificial respiration was begun The cerebellar electrical activity was picked up by means of tungsten microelectrodes The tip diameter of the microelectrodes was about 3–6 μ they were introduced into the cerebellum by means of a microcontrol The microelectrodes were connected through a Grass high impedance probe 5A and a P5CR preamplifier with the lower beam of a Tektronix 502 A oscilloscope

Simultaneously the electrical activity of an oculomotor nerve was recorded by means of another microelectrode connected through a Grass P5CR preamplifier with the upper beam of the oscilloscope In this way the simultaneous record of the cerebellar electrical activity and of the action potentials of a few fibers of an oculomotor nerve gave the possibility to correlate the cerebellar effects of the labyrinthine stimulation to the eye

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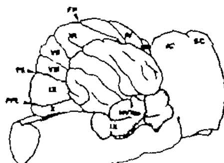
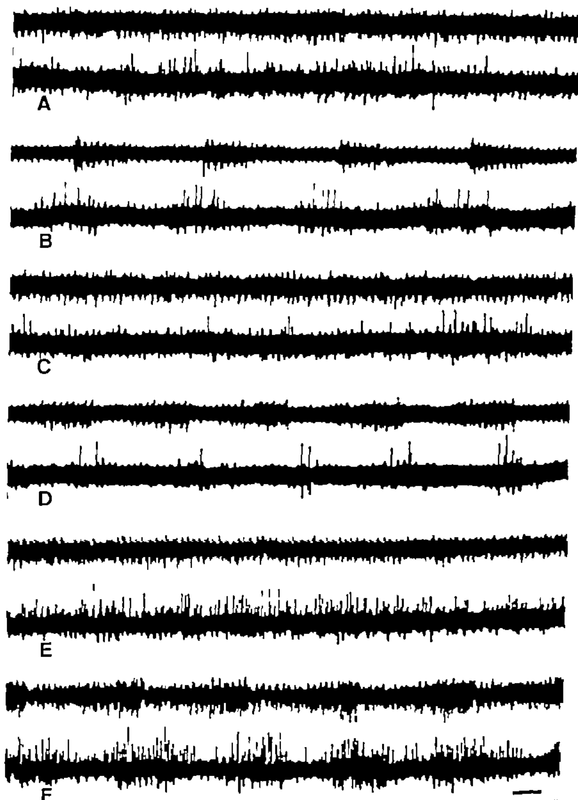


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F a. 2. Simultaneou record of the lectric l a tl ity of the l ft ocul m t r nerv (*pper beam*) nd f th un t ry discharge of th right half of tl e u ula (*l wer beam*) Guinea pig no 490 (A) N rm l ctl ity i resti g conditi ns. (*B*) Immediat ly fter a warm stimul tion f the left labyrinth not th hythmi al q ick responses n the nerv preceded by l w responses of the cer bellar ort x (*C*) Reco ery (*D*) Immedi-

fications of the electrical activity of cerebellar cortex consisting either of tonic or of rhythmic responses

1. Tonic responses: they were characterized by a long lasting activation of the cerebellar units which exhibited an increase in the discharge rate and recruitment of new units before silent, without signs of rhythm (Fig. 3 *B*).

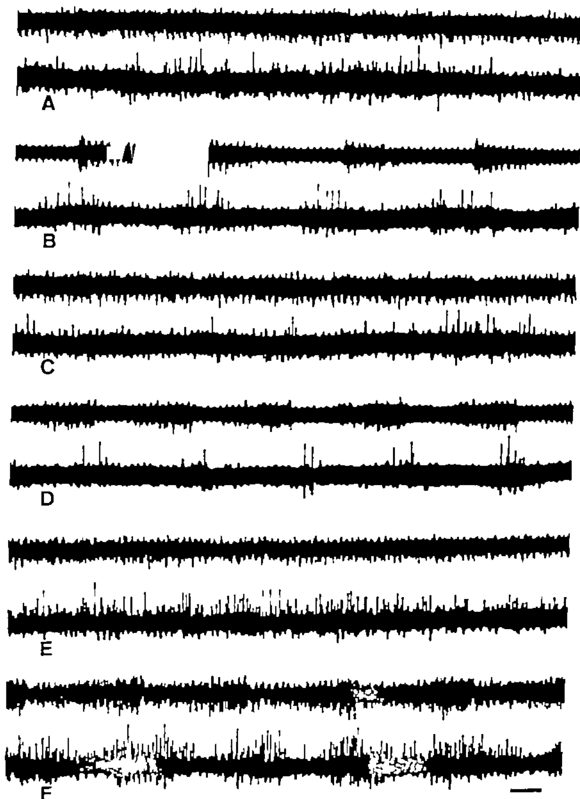
2. Rhythmic responses: *they were characterized by the appearance of* rhythmical outbursts of the electrical activity with intercalated periods of inhibition.

In some cases the rhythmical responses consisted of sudden, asynchronous outbursts of a few units which lasted only 30–35 msec, each outburst being separated either from the preceding or from the following by a period of inhibition of the electrical activity which could last from 200 msec up to 1–2 sec. (*Fig. 2 D*). *These rhythmical responses* looked like the quick rhythmical responses recorded from the oculomotor nuclei (Azzena, 1966; Azzena & Giretti, 1967; Azzena *et al.*, 1967; Manni *et al.* 1965; Manni & Desole, 1966). In other cases the rhythmical outbursts were characterized by a longer duration (from 150 msec to two sec) and consisted of a progressive increase of the discharge rate with recruitment of other units. Short lasting (40 msec) periods of inhibition separated the single outbursts (Figs. 2 *B*, *F*; 3 *C*). Such type of response was similar to the slow responses of the oculomotor nuclei (Azzena, 1966; Azzena & Giretti, 1967; Azzena *et al.* 1967; *Manni et al.*, 1965; *Manni et al.* 1965; *Manni & Desole, 1966*). It is to be noted that no rhythmical responses were recorded from the other cerebellar lamellae and deep cerebellar structures.

Analysis of the combination of the responses of the oculomotor nerve and of the cerebellum to the labyrinthine stimulation

The cerebellar cortex and the oculomotor nerve exhibited various combinations of the above described responses to the labyrinthine stimulation. A tonic discharge *could precede or follow the rhythmical outbursts either* on the nerve or on the cerebellar cortex (Fig. 3 *B*). On the other hand a quick rhythmical response could be shifted to a slow one or vice versa by changing the side of stimulation. This occurred in about / of the rhythmical responses, in which the warm stimulation of a labyrinth induced quick responses of the ipsilateral cerebellar cortex while the stimulation of the contralateral labyrinth elicited slow responses (Fig. 2 *B*, *D*). In the remaining / of the rhythmical responses only slow responses were observed

diately after warm stimulation of the right labyrinth. The oculomotor nerve exhibited slow responses followed by quick outbursts of the cerebellar cortex. (*E*, *F*) Guinea pig no. 478. *E*, normal activity in resting conditions. *F*, immediately after warm stimulation of the left labyrinth. The slow responses of the cerebellar cortex (uvula) began on the final part of the slow responses of the oculomotor nerve and continued during the quick inhibition of the nerve. Calibration 100 msec.



F a. 2. Simult neo rec d f the lectrical ti ity of th l ft ocul m t n (*uppe beam*) and f th t ry discharg of th ight half i the ul (*l me be m*) Guine pig no. 490 (*A*) N m l cti ity resti g ndit (*B*) Immedi t l ft a warm timulation f th l ft l b rinth not the hythmical quick responses on th nerv preceded by l w respons f the cerebell r cort (*C*) Reco ery (*D*) Immedi

On the other hand, if we take into account only the frequency of the rhythmical outbursts of the cerebellum and of the oculomotor nerve, the rhythmical responses can be collected in two different groups

Group 1 This group was characterized by the fact that the rhythmical responses exhibited the same frequency on the cerebellum and on the oculomotor nerve. Some 81% of the recorded sites belonged to this group. In the majority of the cases both the cerebellum and the oculomotor nerve exhibited the same type of response for example, slow responses. On the other hand, in several experiments the nerve exhibited slow responses and the cerebellum quick ones or vice versa. However it is to be noted that the rhythmical outbursts exhibited *by the cerebellum were not synchronous* with those presented by the oculomotor nerve. In fact, the cerebellar out bursts were consistently delayed in relationship to the phases of the eye nystagmus. (Figs. 2 *B D F* 3 *C*)

Group 2. In the remaining 19% of the experiments the rhythmical cerebellar responses *to the thermic stimulation* of the labyrinth showed a frequency which differed from that presented by the rhythmical responses of the oculomotor nerve. The outbursts of the cerebellum were either more frequent or slower than those of the oculomotor nerve.

DISCUSSION

The result of the present investigation provided evidence that ampullar *labyrinthine stimulation* provoked *the appearance of rhythmical responses* on particular cerebellar areas.

Thus the conclusion may be reached that the cerebellum although not essential for the appearance of the eye nystagmus, is the seat of rhythmical activity when ocular nystagmus is present. Several investigators have point ed out that messages from muscles and joints arrive at the cerebellum (Dow & Moruzzi, 1958 Jansen & Brodal, 1954 Snider & Stowell, 1944) However the guinea pigs employed in this research were curarized and no proprioceptive impulses *from the extra-ocular muscles during eye nystagmus* could reach the brain-stem and the cerebellum. Thus it seems more reasonable to relate such rhythmical cerebellar activations to the ampullar receptors in the vestibular nuclei and the brain-stem reticular formation. It is a well known fact that other sensory organs (eye, hearing, skin) project on the cerebellum (Dow & Moruzzi, 1958 Jansen & Brodal, 1954 Snider & Stowell, 1944) The flocculus, paraflocculus and uvula were the cerebellar areas from which the rhythmical outbursts could be recorded. It is noticeable that such areas have clear-cut relationships to the vestibular apparatus (Brodal *et al* 1962) In fact they receive vestibular afferents and conversely they send fibers to the vestibular nuclei. However no rhythmical responses were recorded *from the nodulus although it should* be enclosed in the vestibular areas of the cerebellum. The absence of nodular rhythmical responses to labyrinthine stimulation supports the view of Jansen & Brodal

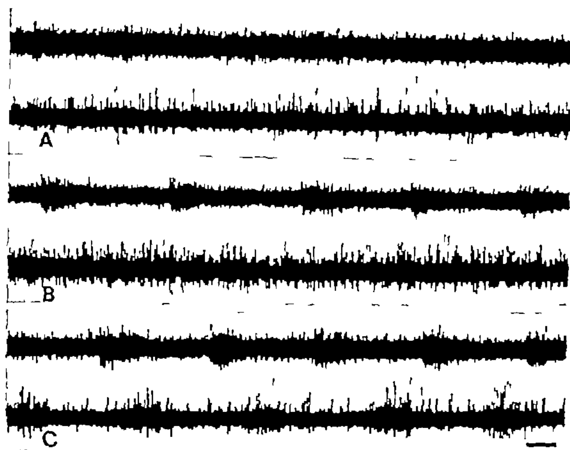


FIG. 1 Simultaneou records of th electrical acti ity of the l ft oculomotor nerve (*upper beam*) and of th u itary discharg of the right h lf of the u ula (*lower beam*) Guinea pig n 481 (*A*) Normal activity in resting onditi ns. (*B*) A warm timulation f the l ft labyrinth induced rhythmical quick responses on the nerve (*B C*) however t th beginning th cerebellar cort x presented toni discharge (*B*) f llowed i *C* by *slow responses during the low inhib tion of the nerve. Calibration 100 msec.*

regardless of the stimulated side A comparison of the response simultaneously recorded from the cerebellar cortex and from the oculomotor nerve offered some difficulty In fact the responses may be differently grouped according to the various criteria of evaluation

By taking into account only the type of response regardless of the stimulated and recorded sides the following combinations were observed

1 In 74% of the stimulations both the cerebellar cortex and the oculomotor nerve exhibited rhythmical responses the responses were slow in 29% and quick in 13% on both cerebellum and 3rd nerve, while in the remaining 32% the rhythmical outbursts were slow on the cerebellum and quick on the nerve or vice versa

2 In 16% of the stimulations rhythmical responses occurred on the oculomotor nerve while the cerebellum presented tonic responses.

3 Finally in the remaining 10% of the stimulations the responses of both the cerebellum and oculomotor nerve were tonic

et al 1958) Thus conclusion may be reached that the cerebellum utilizes messages from the vestibular apparatus in order to influence and regulate the correct performance of the extra-ocular movements just as it acts on the limb movements.

ZUSAMMENFASSUNG

In den curarisierten Meerschweinchen wurde während des Augennystagmus, der durch die termische Reizung des Labyrinths verursacht wird, mit Mikroelektroden die elektrische Aktivität der Kleinhirnrinde, gleichzeitig mit derjenigen weniger Fasern eines Nerves oculomotorius, aufgezeichnet. Der termischen Reizung des Labyrinths folgend, wurden tonische und rhythmische Antworten der Uvula, des Flocculus und des Paraflocculus aufgezeichnet. Die rhythmischen zerebellaren Antworten haben im 81% der Fälle die gleiche Frequenz des Augennystagmus ergeben. Die rhythmischen zerebellaren Antworten waren jedoch im Grossteil der Falle mit der langsamen oder schnellen Fase des Nystagmus nicht perfekt synchron In den übrigen 19% der Fälle war die Frequenz der rhythmischen zerebellaren Antworten von der des Augennystagmus verschieden.

Es ergibt sich deshalb, dass das Kleinhirn, obwohl nicht wesentlich für das *Erscheinen des Aug nnystagmus, Sitz* *hythmischer Aktivität während des Ny*stagmus ist. Auf diese Art benützt das Kleinhirn Botschaften des Vestibularapparates, um die perfekte Ausführung der Augenbewegung zu beeinflussen, wie es für die Bewegung der Glieder handelt.

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(1954) that a functional difference between flocculus and nodulus does exist

By recording simultaneously the action potentials of the cerebellar cortex and those of a few fibers of an oculomotor nerve it was *possible* to correlate the rhythmical cerebellar responses to the phases of the eye nystagmus elicited by the vestibular stimulation Thus in the great majority of the cases (81%) the *rhythmical* outbursts recorded from the cerebellum exhibited the same frequency as that presented by the eye nystagmus However only in a few cases were the rhythmical cerebellar responses perfectly synchronous with the phases of the eye nystagmus frequently the *cerebellar* responses were slightly delayed in relationship to the quick or slow phases of the eye nystagmus. One could suppose that the impulses travelling from the vestibular nuclei and from the reticular bulbo-ponto-mesencephalic formation towards the cerebellum are mediated by chains of intercalated neurons. In *fact* Lorente de Nó (1933) and Horcholle & Tyc Dumont (1968) pointed out that the mechanism of the eye nystagmus involves several chains of neurons in the brain stem It is more difficult to find an explanation for the cases in which the cerebellar responses exhibited a clear-cut different frequency from that of the eye nystagmus. Recently also Manni & Giretti (1968) observed that the rhythmical outbursts exhibited by the vestibular nuclei after labyrinthine stimulation were not synchronous with the phases of the eye nystagmus in some instances the vestibular nuclei showed a higher frequency than the eye nystagmus. By taking into account the connections of vestibular nuclei with the cerebellar areas one could suppose that such types of rhythmical cerebellar responses were driven by the vestibular nuclei

It is to be noted that the rhythmical responses of the cerebellum to the labyrinthine stimulation escaped the attention of the other researchers who analyzed the electrical activity of the cerebellum during labyrinthine stimulation in mammals In fact only tonic cerebellar responses were observed (Arduini & Pompeiano 1957 Camis, 1910 Price & Spiegel 1937 Riva Sanseverino & Urbano, 1965 Weber & Steiner 1965) Only Gualtierotti *et al* (1958, 1959) pointed out the relationships of cerebellar rhythmical outbursts to the nystagmus of the pigeon head during and after angular accelerations of the animal However no rhythmical responses were reported in the experiments carried out by Gualtierotti & Passerini (1958) in the cat they related the cerebellar electrical activity of the cat to the action potentials of the neck muscles and they did not take into account the electrical activities of the extra-ocular muscles or of the oculomotor nerve

Now a question arises what is the functional meaning of such cerebellar rhythmical activity in relationship to the eye nystagmus It is to be noted that alterations of the extra-ocular movements were observed in human beings with cerebellar diseases as well as in totally cerebellectomized animals there was delay and slow contraction, hypermetria, oscillation in the movements and in maintaining the position of the eyes (Alajouanine

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ACTIVE ION TRANSPORT FROM THE SCALA VESTIBULI INTO THE SCALA MEDIA

Participation therein of Reissner's Membrane and its Blockade

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The effect of G-strophantin and 2.4-dinitrophenol introduced into the scala vestibuli of 24 guinea pigs was studied by measuring endocochlea and microphonic potentials (EP and MP) which indicate ion transport and the activity of hair ells. MP were evoked by tones of 500 1000 and 2000 c/sec. and an intensity of 60 dB. MP were always found to change together with EP but n dependence on changes of EP Inhibition of ion transport with G-strophantin introduced into the scala vestibuli decreased EP t nearly ero while 2.4-dinitrophenol even caused EP to show negati e alues which in part represent the diffusion potential of certain ionic difference between the cochlear duct and the surroundings. It is concluded that t nly the stria vascularis, but also Reissner's membrane, participate in acti e i n transport between th cochlear duct and ts surroundings. The active ion transport is a source f EP

In recent years increased attention has been paid to electrophysiological and biochemical changes in the cochlear duct and to the origin of the endolymph. Békésy in 1952 described endocochlear potentials (EP) in the cochlear ductus between +60 and +80 mV Smith *et al* (1954) demonstrated that the compositi n of the end lymph differed from that of other extracellular fluids. In the guinea pig it has a high level of K (144 meq/l) and a low level of Na (15.8 meq/l) while the composition of the perilymph is the opposite (150 meq/l Na and 4.8 meq/l K) Similar values were later obtained f r man (Citron *et al* 1956 Rauch & Kostlin, 1958 Hladký & Opletal, 1965) Smith *t al* (1958) found the K and Na concentration to be the same in the whole endolymphatic ystem although the EP have different values in different parts f the membranaceous labyrinth cochlear duct +80 mV sacculus −1 mV utriculus +4 mV and ampulla −1 mV and this indicates that EP alue do not depend on the concentrations of salts in different part of th labyrinth.

Tasaki *t al* (1954) Da is (1957) Tasaki & Spyropoulos (1959) and Vosteen (1961) believe that the stria vascularis is the source of EP and con ider Reissner s membrane to be an effective insulator Rauch (1962,

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Tasaki *et al* (1954) Davis (1957) Tasaki & Spyropoulos (1959) and Vosteen (1961) believe that the stria vascularis is the source of EP and consider Rei sner's membrane to be *an effective* insulator Rauch (1962,

1963, 1966) on the other hand, and Choo & Tabowitz (1964) demonstrated rapid transfer of ^{24}Na and ^{42}K from the scala vestibuli into the scala media. K was found to be transported three times as rapidly as Na from the perilymph into the endolymph although K is transported against a greater concentration gradient. Active ion transport by cells and the origin of membrane potentials have been studied extensively (Ussing, 1948 Harris & Maizels, 1951 Steinbach, 1952 Hodgkin & Keynes, 1955 Janáček, 1963)

Substances inhibiting active transport include G strophantin and 2,4-dinitrophenol Schatzmann (1953) explains the inhibitory effect of G-strophantin by a direct action on transport activity without there being an effect on total cell energy metabolism The blocking effect of 2,4-dinitrophenol is due to inhibition of ATP formation which is the energy source for active ion transport.

These findings led us to inquire how far EP depend on the activity of cells of Reissner's membrane This touches problems of the origin of the endolymph, metabolism in the inner ear and the origin of cochlear potentials

METHOD

A total of 24 hearing guinea pigs weighing 250–300 g was anaesthetized with 0.8 ml/100 g body weight 20% urethane. EP and microphonic potentials (MP) were recorded after opening the bulla on the ventral side and introducing glass microelectrodes with a micromanipulator through the stria vascularis into the scala media in the 2nd coil Glass microelectrodes with a resistance of 4–7 M ohm and were filled with 3 M KCL connected via a chlorinated Ag wire across a cathode-ray follower to a direct-current amplifier Amplified cochlear potentials were recorded with an ink recorder and controlled on an oscillograph

For MP determinations a tone of 500 1000 and 2000 c/sec and 60 dB intensity was applied for one sec. The intensity of the sound was approximately determined at the entry into the meatus with an artificial recording ear

For perfusions the scala vestibuli was opened at the basilar coil and at the top of the labyrinth with a fine drill Into the basilar coil a fine cannula was introduced which led the perfusion fluid into the scala vestibuli. The fluid left the apex for the bulla and was there collected with cotton wool tampons (Fig 1) The rate of perfusion was such that the pressure of the fluid did not cause changes in the endocochlear potentials. In group I (control) only Ringer's solution was used, in group II 8.5 10^{-5} M G-strophantin in Ringer's solution and in group III 1 10^{-3} M 2,4-dinitrophenol previously neutralized also in Ringer's solution

At the start of the experiment lasting 60 min, initial values of EP and MP were determined. In the 5th min the perfusion cannula was introduced Between the 10th and 15th min the scala vestibuli was perfused with the chosen solution All EP and MP values were continuously recorded For

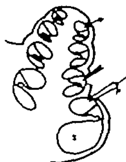


FIG. 1

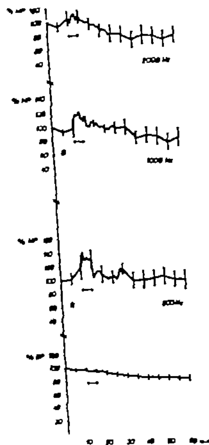


FIG. 2

FIG. 1 Schema of perfusion of the scala vestibuli. S.T scala tympani; S.V scala vestibuli; P cannula, inserted i S.V; M microelectrode inserted in scala media f the 2nd cochlear coil

FIG. 2. Effect of perfusi the scal estibul with Ringer' solution on EP and MP A MP esponse in % t tone of 2000 /sec B, the same t 1000 /sec; C, the same to 500 / sec D endocochlear potentials i % ↔ time f perf si of scala estibuli; [D

statistical ev luation alues found at 5-minute intervals, and between the 10th and 45th minute one-minute intervals were used. Because of the scatter of alues all results had to be calculated as percentages so that they could be compared—except EP in group III The mean value from deter minations at the tart of the experiment after opening the scala vestibuli was t ken as 100

In group III EP alues fell below zero after 2.4-dinitrophenol application and thu their n gative value was due in addition to the generated positi e EP lso to the diffusion potential which is negative and due to the dif f rence in the concentration of ions in the scala media and its surroundings. Here absolut alues were used to express the relationship more exactly

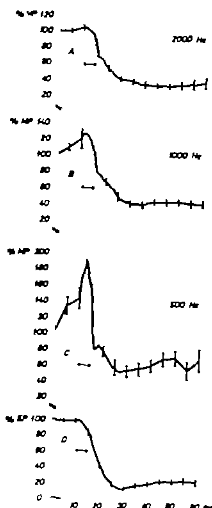


Fig 3

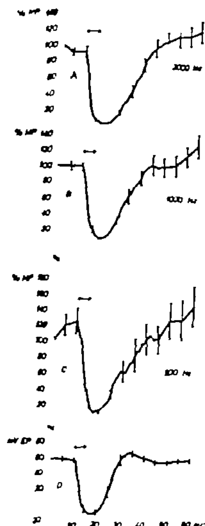


Fig 4

Fig. 3 Effect of perfusion on scala vestibuli with G stropha tin n EP and MP A MP response in % to tone of 2000 c/sec B the same to 1000 c/sec C, the same t 500 c/sec D endocochlear potentials in % ↔ tim of perfu i of scala vestibuli ⟂ s.D.

Fig 4 Effect of perfusion n scala vestib li with 2,4-dinitrophenol n MP a d EP A MP response in % to tone of 2000 c/sec B the same to 1000 sec C the same to 500 c/sec D endocochlear potential in ⟵⟶ time of perf n of scala vestibuli ⟂ s.D.

All data were evaluated statistically at the given time intervals. Figures were prepared from the arithmetic means and standard deviations. The student s t test was used

RESULTS

In group I (control—Fig 2 *A B C D*) the scala vestibuli was perfused from the 10th to the 15th minute Perfusion did not cause changes in the EP (Fig 2 *D*) In the 4th coil (corresponding to tones of 500 c/sec) MP values were significantly increased during perfusion (Fig 2 *C*) In coil 2 and 3 (tones 1000 and 2000 c/sec) no effect was seen (Fig 2 *B A*)

In group II perfusion with G-strophantin caused a fall in EP values, significant from the 6th minute after the start of perfusion to the end of the experiment. The largest decrease (to 11% ±2.04) was found in the 30th minute (Fig. 3 *D*) The initial rise in MP values was due to application of the cannula to the opening of the basilar coil of the scala vestibuli before perfusion. This factor affected particularly the frequency in the 4th coil (500 c/sec, Fig. 3 *C*) and partly also in the 3rd coil (1000 c/sec Fig 3 *B*) Within 3 min after the start of perfusion, MP increased slightly and this was significant particularly in coil 4 (500 c/sec) Then they started to fall so that there was a statistically significant decrease between the 6th minute of perfusion and the end of the experiment. The lowest MP values were found in the 28th minute of the experiment (Fig. 3 *A B C*)

In group III (Fig 4 *A B C D*) perfusion with 2 4-dinitrophenol caused a pronounced fall in EP which was significantly different from group I in the 11th minute of the experiment, i.e. 3 min after start of perfusion and which fell to negative values in the 14th minute (Fig 4 *D*)

The largest fall in EP to −13.6 mV ±1 02 was found in 18th minute. Then EP started to rise again, so that in the 31st minute initial values were again obtained. The MP values fell in the 2nd, 3rd and 4th coil at the same time (Fig 4 A B C) The largest fall was again in the 18th minute where MP attained zero values, to 10% of the average initial values. Since the effect of 2.4-dinitrophenol is short-lasting, MP started to rise after the 18th minute and reached initial values in the 40th minute.

Thus both G-strophantin and 2.4-dinitrophenol caused a *fall in EP and* MP while Ringer's solution itself had no such effect.

G-strophantin maintained decreased EP up to the end of the experiment but zero values were not attained. 2.4-dinitrophenol, on the other hand, caused a rapid fall in EP down to negative values. Its effect was short lasting and hence in the 31st minute values returned to normal.

MP values were always found to alter together with EP values. It may be concluded that Reissner's membrane which separates the space of the scala vestibuli from the scala media is not completely impermeable to ions and that its cells participate in the ionic exchange between these spaces.

DISCUSSION

Naftalin & Harrison (1958) first applied the finding of Hodgkin & Keynes (1955) on membrane potential to the inner ear They put forward the hypothesis that ions pass through the Reissner's membrane. Rauch & Kostlin (1962, 1963) and Choo & Tabowitz (1964) showed that labelled Na and K pass through this membrane and this is *also* indicated by electronmicroscopic work. Lawrence *et al* (1961) Iurato (1967) Duval (1967) showed that the layer of epithelial cells of the Reissner's membrane facing the ductus cochlearis contain many mitochondria and other particles. The inner surface of these cells has many microvilli. Adenosine triphosphatase activity

has also been demonstrated on the surface of the endolymphatic area of the membrane and this enzyme is found at sites of active transport Nakai & Hilding (1967) Ussing's work (1960) and that of others shows that active ionic transport across membranes depends on respiration or glycolysis (mammalian red blood cells) since inhibition of these processes or anoxia of the whole animal leads to a fall in ion transport Reissner's membrane also behaved thus in decapitated animals (Rauch) Inhibition with G strophantin, however of ionic transport has no effect on oxygen consumption and glycolysis (Schatzmann, 1953) but influences ion transport mechanism.

It must be considered whether G-strophantin and 2 4-dinitrophenol can not block ionic transport from the perilymph into endolymph across the tissue of the lateral wall, i c. via the spiral ligament into the stria vascularis. Tonndorf *et al* (1962) investigated the propagation of various dyes after their application into the scala vestibuli and they found that the Reissner's membrane is relatively impermeable. It is therefore assumed that ions are transported from the scala vestibuli into the scala media via the spiral ligament and the stria vascularis.

It is well known that 2 4-dinitrophenol and G-strophantin block the active ion transport. After the application of these substances into the scala vestibuli the EP decreased almost to zero which proves that EP is generated by the active ion transport According to Tasaki & Spyropoulos (1959) the stria vascularis represents the source of EP whereas the findings of Lawrence *et al* (1961) Duval (1967) Nakai & Hilding (1967) and Iurato (1967) support our view that the Reissner's membrane also partly participates in the active ion transport

Davis *et al* (1955) found that the EP depend more on oxidative mechanisms than on the ionic difference in the surroundings medium This does not contradict our findings. The active ionic transport described here is dependent on aerobic or anaerobic processes. Davis (1957) considers the wall of the cochlear duct to be practically impermeable to ions This conclusion is based on the work of Békésy (1951) who found that the wall of ductus cochlearis is very resistant to the direct current and to the low frequencies of alternating current Rauch (1964) on the other hand, states that no membrane of the living cells with a relatively high metabolic rate is impermeable or nearly impermeable to water and ions and also demonstrates this. Chou (1963) showed by respirometry that Reissner's membrane has the same rate of oxygen consumption as the stria vascularis and values are among the highest found in the animal Honrubia *et al* (1965) showed that perfusion of the scala vestibuli in anoxaemic guinea pigs maintains the EP at normal values while perfusion of the scala tympani has no effect on the fall in EP due to anoxia Thus again Reissner's membrane seems to be permeable in these experiments.

Our data on the rapid inhibition of EP and MP by the drugs used agree with those of Rauch *et al* on ion transport across Reissner's membrane and in addition show that EP are generated by active transport of ions.

ZUSAMMENFASSUNG

Bei 24 Meerschweinchen wurde der Einfluss von G-Strophantin und 2,4-Dinitrophenol nach deren Applikation in die Scala vestibuli auf den aktiven Ionentransport durch Messung der endocochlearen und mikrophonen Potentiale (EP und MP) ermittelt. Die MP wurden mittels Tönen von 500, 1000 und 2000 Hz bei einer Intensität von 60 dB untersucht. In allen Versuchen wurde beobachtet, dass die MP immer parallel in Abhängigkeit v n den Veränderungen der EP sanken oder anstiegen.

Es wurde festgest llt, dass die Blockade des Ionentransportes durch di Applikation on G-Strophantin in die Scala vestibuli inen Ruckgang des EP beinahe bis auf *einen Nullwert verursacht*. Die Applikation von 2,4-Din trophenol in die Scala estibuli hatte sogar einen Ruckgang der positiven EP Werte bis zu negativen Werten zur Folge, wo die Messwerte einen Teil des diffusen, durch die Ionendifferenz zwischen dem Cochlear duct und der Umgebung bestimmten Potentials orstellen.

Aus diesen Ergebnissen wird darauf geurteilt, dass an dem Ionenaustausch zwischen dem Ductus cochlearis und der Umgebung ni ht nur die Stria vascularis, *w e bereits früher nachgewiesen wurde sondern* och die *Reissnermembran* beteiligt ist. Der aktive Ionentransport bildet eine Quelle des EP

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DAS FREQUENZMAXIMUM DES KALORISCH AUSGELÖSTEN NYSTAGMUS I ALS KENNLINIENFUNKTION DES GEPRÜFTEN VESTIBULARORGANES

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Die Schlagfrequenz des postkalorischen Nystagmus I, welche aussagekräftiger ist als die Dauer, wird in dieser Arbeit zur Beurteilung des geprüften Vestibularorganes dergestalt benutzt, daß aus der Schlagrate während des 30-Sekunden Bereiches maximaler Schlagfrequenz ein Kennlinienzeiger abgeleitet wird. Bei entsprechender Anordnung der vier Kennlinien der Warm- und Kaltprüfung beider Ohren in den vier Quadranten eines rechtwinkligen Koordinatensystemes kommt man zu einer Zeigerdarstellung, die eine vereinfachte Beurteilung der Ergebnisse der kalorischen Vestibularisprüfung ohne ENG und PENG zuläßt. Durch entsprechende Konstellationen der Kennlinienzeiger erhält man einprägsame typische Muster für z.B. periphere vestibuläre Seitendifferenzen, nystaktisches Richtungsüberwiegen, Spontannystagmus, Verlaufskontrolle etc. Die Anwendbarkeit dieser Methode wurde bei 126 kalorischen Vestibularisprüfungen erprobt.

Diese Arbeit will versuchen, dem praktisch tätigen Otoneurologen auch ohne Elektronystagmographie und Photoelektronystagmographie allein mit der Frenzel-Brille und einer Stoppuhr oder einem einfachen Zeitkurvenschreiber eine Möglichkeit an die Hand zu geben, durch eine entsprechende Auswahl und Anordnung seiner Beobachtungen relativ schnell zu einer quantitativen Aussage über den postkalorischen Nystagmus I zu kommen, die an Aussagekraft das bisher übliche Schema von Hallpike (Fitzgerald & Hallpike, 1942) übertrifft, welches die Nystagmusdauer als Grundlage benutzt.

Ein Parameter der Vestibularisfunktion, der für jeden einzelnen kalorischen Reiz auch eine Reizantwort nach Art einer Häufigkeitsverteilung (ähnlich der Normalverteilung nach Gauss) liefert (Hinchcliffe, 1967; Torok, 1967) ist die Frequenzverteilung des postkalorischen Nystagmus I. Das Nystagmusfrequenzmaximum ist besonders leicht beobachtbar, da sich zu diesem Zeitpunkt auch die Amplitude im Maximalbereich befindet. Das Integral der Frequenzverteilung über der Zeit ergibt eine kumulative Verteilung, die aus der Technik als sog. Kennlinie eines geprüften Prozesses oder eines gesteuerten Systems bekannt ist. Zum ist Verlaufen derartige

Kennlinien S-förmig, wobei der zentrale Bereich dieser S-Kurve *mehr oder* weniger weit asymptotisch einer Geraden angepasst ist, die als Tangente an den Wendepunkt bekannt ist Die Steigung dieser Tangente ist ein bewährter Index zur Beurteilung der Leistungsdynamik des geprüften Systems. Ein besonderer Vorteil dieser Betrachtungsweise beruht darauf dass bei der statistischen Untersuchung einer Haufigkeitsverteilung in Form der Kennlinie vom Zentrum weiter entfernte, oft schwerer messbare Rand werte vernachlässigt werden können. *Gerade diese Werte können die* genaue Festlegung der bisher gebräuchlichen Nystagmusdauer behindern, die ohnehin am aussageschwächsten unter den Nystagmusparametern Dauer Frequenz und Amplitude steht (Henriksson *et al* 1967 Mittermaier 1965 Torok, 1957) Als ein maximales Erregungsmuster des geprüften Vestibu larorganes könnte man die Kulmination der postkalorischen Schlagrate bezeichnen. Diese Tatsache wurde auch von Hinchcliffe (1967) besonders *herausgestellt Wir er nachweisen konnte besteht eine hohe Korrelation* zwischen der Geschwindigkeit der langsamen Phase im Elektronystagmogramm und der Frequenz in einem 30-Sekunden Intervall um den Kulmina tionspunkt herum. Die Frequenzverteilung soll in diesem Bereich einer Gauss schen Verteilung entsprechen

Besonders günstig für den Beobachter verhält es sich, dass die maximale Nystagmusfrequenz nur sehr selten fünf Schläge/sec erreicht oder sogar *höher liegt Fünf Schläge pro sec kann der geübte Beobachter aber z. B.* mit der Eichtaste eines kleinen EKG-Gerates einer Zeitkurve aufdrucken oder nach Zeitklassen geordnet mitzahlen

Begrenzt man den zentralen Bereich der oben genannten S-förmigen Kennlinie zeitlich im Bereich des angenähert geradlinigen Verlaufes, so entspricht die Steigung dieser angenäherten Geraden dem Tangens des Quotienten von abgegrenzten Einzelschlägen zu gleichermassen abgegrenz *ten Sekunden Wählt man entsprechend den statistischen Versuchserfah* rungen ein stets gleichgrosses Zeitintervall zum Zählen der Nystagmus schlage wahrend der Frequenzkumulation, dann lässt sich diese Schlagrate so in einem rechtwinkligen Koordinatenschema abbilden, dass die Kenn linienfunktion als Zeiger zur Darstellung kommt (Abb 1)

Empirisch konnte entsprechend den Ergebnissen von 136 postkalorischen Frequenzzeitkurven die vom Verfasser im klinischen Routinebetrieb mit *Frenzel* Brille und Eichtaste eines Zeitschreibers ermittelt wurden der Zeit raum der bei der Zeigerdarstellung verwendeten Frequenz auf 30 sec begrenzt werden Im Zentrum dieser 30-Sekunden Strecke befinden sich die leicht auffindbaren 10 Sekunden höchster postkalorischer Nystagmusfrequenz, hinzugefügt wurden die beidseits benachbarten 10-Sekunden, um dem er haltenen Wert eine breitere Basis zu geben Zur graphischen Ermittlung der Kennlinie werden auf der Abszisse eines rechtwinkligen Koordinaten systemes die Einzelsekunde und auf der Ordinaten der Einzelschlag als gleichgrosse Einheiten gewahlt Da nun bei allen Versuchen ein immer gleicher Zeitraum zugrunde gelegt wird namlich die zentralen 30 Sekun

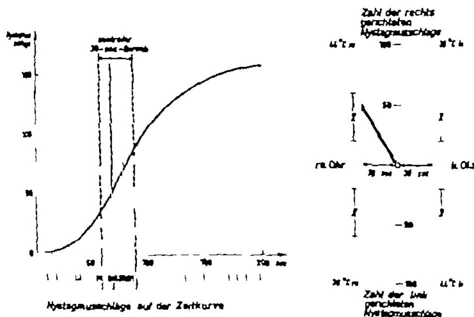


Abb. 1

den, kann man stets eine Ordinaten-Parallele durch den 30-Sekundenpunkt der Abszisse benutzen, um auf ihr die gefundene Schlagzahl während des zentralen 30-Sekundenbereiches aufzutragen. Verbindet man den Schlagzahlpunkt mit dem Nullpunkt des Koordinatensystemes, so hat man die Kennlinie für das postkalorische Frequenzverhalten annähernd festgelegt (Abb. 1)

Die vier Kennlinienzeiger die man ermittelt, nachdem nacheinander beidseits 30 sec lang mit je 20 ml 44 Grad und 30 Grad warmen Wassers gespült wurde lassen sich so auf die vier Quadranten eines rechtwinkligen Koordinatensystems verteilen, dass links von der Ordinaten die Ergebnisse der kalorischen Prüfung des rechten Ohres, rechts von der Ordinaten die Ergebnisse der kalorischen Prüfung des linken Ohres, oberhalb der Abszisse die Ergebnisse bei rechtsgerichtetem Nystagmus und unterhalb der Abszisse die Ergebnisse bei linksgerichtetem Nystagmus angeordnet werden d. h. im I Quadranten wird die Kennlinie nach Kaltspülung links, im II Quadranten die Kennlinie nach Warmspülung rechts, im III Quadranten die Kennlinie nach Kaltspülung rechts und im IV Quadranten die Kennlinie nach Warmspülung links ermittelt (Abb. 1)

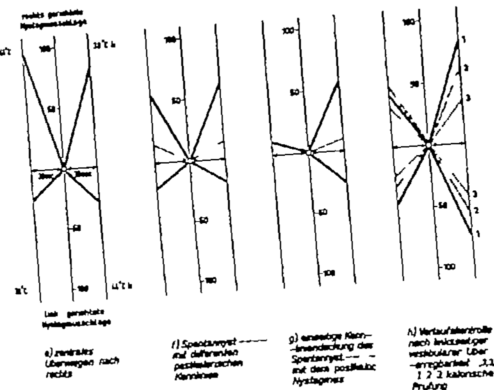
Markiert man sich an der schlagratenzählenden Ordinatenparallele noch die mittlere Schlagrate ± der Standardabweichung als Maß der Streuung — bei unseren 136 kalorischen Prüfungen betrug die mittlere Schlagrate der zentralen 30 Sekunden 40 ± 20 Schläge — so kann auch der weniger Geübte die komplexen Ergebnisse der 4 zusammenhängenden kalorischen Vestibularprüfungen beurteilen.

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e) zentrales Überwiegen nach rechts

f) Spontannyst. ----- mit differenten postkalorischen Kennlinien

g) einseitige Kennlinienablenkung des Spontannyst. --- mit dem postkalor. Nystagmus

h) Verlaufskontrolle nach linksseitiger vestibulärer Übererregbarkeit 3,2,1 1 2 3 kalorische Prüfung

$\bar{X} \pm s$

Abb. 3.

4 Nystaktisches Richtungsüberwiegen.
(*a*) Verstärkung einer Nystagmusrichtung — die Maximumschlagraten der Warmprüfung eines Ohres und der Kaltprüfung des kontralateralen Ohres überschreiten den Normbereich (Abb 3 *e*)
(*b*) Unterdrückung einer Nystagmusrichtung — die Maximumschlagraten der Warmprüfung eines Ohres und der Kaltprüfung des kontralateralen Ohre unterschreiten den Normbereich.

5 Überlagerung durch einen Spontannystagmus. Die Frequenz des Spontannystagmus wird vor der kalorischen Prüfung über 30 sec ausgezählt, die beiden erhaltenen Schlagraten werden in demselben Schema in zwei Quadranten abgetragen, so dass zwei richtungsentsprechende Kennlinienzeiger entstehen. Anschliessend werden in demselben Schema die Ergebnisse der kalorischen Prüfung eingetragen. Beim Vergleich des postkalorischen und des Spontannystagmus ergeben sich folgende Möglichkeiten
(*a*) Kennliniendifferenz mit Verstärkung des dem Spontannystagmus gleichgerichteten postkalorischen Nystagmus (Abb. 3 *f*)

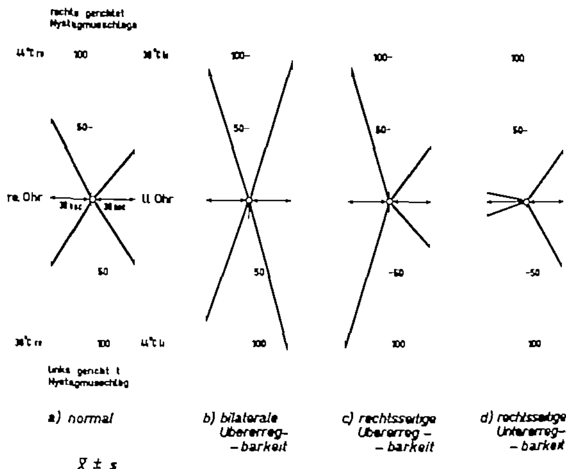


ABB. 2.

Teils als Ergebnis unserer Untersuchungen und teils aufgrund theoretischer Überlegungen entstehen bei Anwendung der oben beschriebenen Methode typische optisch einprägsame Muster die entweder rein oder als Mischformen auftreten können. Diese Muster sollen im folgenden schematisch zusammengestellt werden

1 Normale kalorische Erregbarkeit beider Vestibularorgane. Die Maximum Schlagraten befinden sich für alle vier Prüfungen im Normbereich (Abb 2 *a*)

2 Bilaterale Erregbarkeitsveränderungen

(*a*) bilaterale Übererregbarkeit — die Maximumschlagrate überschreitet allseitig den Normbereich (Abb 2 *b*)

(*b*) bilaterale Untererregbarkeit — die Maximumschlagrate unterschreitet allseitig den Normbereich

3 Periphere Seitendifferenzen

(*a*) einseitige Übererregbarkeit — die Maximumschlagraten der Warm und Kaltprüfung eines Ohres überschreiten den Normbereich (Abb 2 *c*)

(*b*) einseitige Untererregbarkeit — die Maximumschlagraten der Warm und Kaltprüfung eines Ohres unterschreiten den Normbereich (Abb 2 *d*)

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(*b*) Kennliniendifferenz mit Unterdrückung des dem Spontannystagmus entgegengerichteten postkalorischen Nystagmus (Abb 3 *f*)

(*c*) einseitige Kennliniendeckung des Spontannystagmus und des gleichgerichteten postkalorischen Nystagmus einer Seite ohne Richtungsumkehr des Spontannystagmus *bei entgegensetzter kalorischer* Prüfung auf der gleichen Seite (Abb 3 *g*) (Meist treten (*a*) und (*b*) vergesellschaftet auf!)

6 Vergleich mehrerer Untersuchungen an demselben Patienten

(*a*) Verlaufskontrolle mittels der Kennlinienzeigerbewegung bei Ausbildung oder Rückbildung einer der obengenannten Störungen (Abb 3 *h*)

(*b*) Adaptationstest nach Henriksson *et al* (1961) Kennlinienvergleich der 1., 2., 3., und der *n* ten kalorischen Prüfung desselben Patienten am gleichen Tage.

Aussagen über den Erfolg kalorischer Reizung bei Vorliegen eines Spontannystagmus lassen sich nach der üblichen Hallpike-Methode nur treffen, wenn es zu einer Nystagmusumkehr kommt während mit dieser Methode quantitative Unterschiede zwischen dem gleichgerichteten spontanen und postkalorischen Nystagmus herausgearbeitet werden konnen.

Technisch bessere Ergebnisse als das reine Abschätzen des Maximumbereiches und sein Auszählen nach der Stoppuhr liefert das Aufdrucken der einzelnen beobachteten Nystagmusschlage auf eine Zeitkurve, die anschließend ausgezahlt wird Bei unseren Versuchen lag das mittlere Maximum der Schlagenfrequenz des postkalorischen Nystagmus I $\pm$ Standardabweichung 77 ± 18 sec nach Spülbeginn Bei Auszählung des Frequenzmaximums nach der Stoppuhr erscheint uns der Bereich zwischen 60–90 Sekunden nach Spülbeginn wichtig

Möglicherweise kann diese Methode auch zur Beurteilung der Geschwindigkeit der mit dem Elektronystagmogramm oder Photoelektronystagmogramm registrierten langsamen und schnellen Phase des postkalorischen Nystagmus I benutzt werden.

SUMMARY

Using a strip-chart recorder and FRENZEL glasses, the frequency of post caloric nystagmus *is manually plotted on the constantly moving chart.* From a sample of 130 cumulative frequency distributions have been constructed according to each frequency strip The important central parts of the S-shaped cumulative distributions approximate linear curves, especially during the central 30 seconds. As a practical application the tangent from the beats on the central 30 seconds easily can be sketched in a rectangular coordinate system. This is then used as an index of one caloric test. *If arranging the indexes from the four tests* of one patient on the four quadrants of the coordinate system the result of the whole calorisation is transformed into a graphical pattern resembling a butter fly Different shapes are associated with different diseases.

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COMBINED ACTION OF LARYNGEAL MUSCLES

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In the lightly halothane anesthetized dog the cricothyroid and posterior crico-arytenoid muscles are active simultaneously With electrical stimulation of the cricothyroids, laryngeal resistance is increased and cross sectional area of the larynx decreased. With electrical stimulation of the posterior crico-arytenoids, laryngeal resistance is decreased and cross sectional area of the larynx increased. Stimulation of the crico-thyroid and posterior crico-arytenoid muscles simultaneously results in an even lower airway resistance and an even larger cross sectional area than the posterior crico-arytenoid muscles can accomplish alone. This synergism is described and illustrated.

The respiratory function of the larynx is that of a variable resistance in the upper airway The changes in resistance are carried out by the co-ordinated activity of intrinsic and extrinsic muscles of the larynx. Through studies of anatomy nerve cutting, nerve and muscle stimulation and electromyography the individual activity of the laryngeal muscles has been described (Nakamura *et al* 1958 Negus, 1962 Brewer *et al* 1962, 1963 Rattenborg *et al* 1963) However laryngeal muscles do not act in an isolated manner In order to understand laryngeal dynamics we must determine how laryngeal muscles act in combination.

From electromyographic studies in the dog, under light halothane anesthesia, we found that the cricothyroid and the posterior crico-arytenoid muscles usually act simultaneously during inspiration The paradox of a closing muscle (cricothyroid) acting simultaneously with an opening muscle (posterior crico-arytenoid) during inspiration (when the larynx opens) suggested further studies (Fig 1)

METHODS

In situ larynx

Four dogs under halothane anesthesia with denervated larynx had electrodes inserted in both cricothyroid and both posterior crico-arytenoid muscles for stimulation. Laryngeal resistance was measured as the pressure drop across the larynx through which a constant airflow was passed

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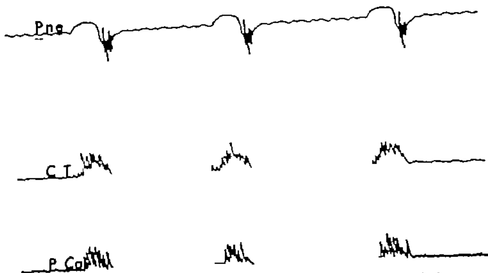


Fig. 1 Pne pneumotachograph curv with inspiratio up CT integrated lectromyograph of the cricothyroid muscle; PCa: integrated electromyograph of the posterior crico-arytenoid muscle

Isolated larynx

The larynx was removed from two anesthetized dogs rapidly electrodes inserted as above and movies taken during stimulation

In both experiments a Dise Multistim was used. A diphasic impulse at 100 cps and 0.2 msec duration was used. Voltage could be varied individually to each stimulating electrode through a rheostat from 0–10 olts.

PROCEDURE

The cricothyroid and posterior crico-arytenoid muscles were stimulated first individually and later simultaneously

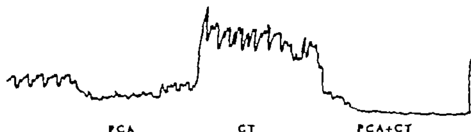


Fig. 2. Tracing of laryngeal resistance during muscle timulation measured as pressure drop across the l ynx d ring constant flow PCA stim lation of posterior cricoaryt noid CT stim lation of cricothyroid PCA nd CT simultaneous stimulation of posteri crico-aryt noid nd cricothyroid.

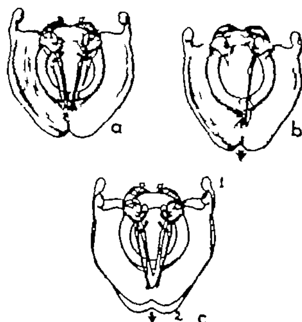


FIG. 3 (*a*) Opening of larynx by posterior crico-arytenoid (*b*) cricothyroid increases AP diameter but glottis passively closed (c) synergistic action of both muscles results in greatest cross sectional area.

RESULTS

Stimulating the cricothyroid muscles individually increased the laryngeal resistance in the *in situ* preparation In the isolated larynx preparation the anterior/posterior diameter increased but vocal cords were approximated causing a decreased cross sectional area.

Stimulating the posterior crico-arytenoids alone reduced laryngeal resistance in the *in situ* preparation and opened the vocal cords in the isolated larynx.

Balanced stimulation of cricothyroids and posterior crico-arytenoids together resulted in the lowest resistance recorded *in situ* and in the greatest cross sectional area in the isolated larynx (Figs. 2 and 3)

DISCUSSION

The apparent paradox of a closing muscle (cricothyroid) and an opening muscle (posterior crico-arytenoid) acting simultaneously during inspiration is resolved by the finding that they can act synergistically to provide greater opening of the larynx than the posterior crico-arytenoids can alone

ZUSAMMENFASSUNG

Die Kehlkopfmuskeln Krikothyroideus und Krikoarytenoideus Posterior sind im Hunde unter Halothane Narkose zur selben Zeit aktiv Während elektrischer Reizung des Krikothyroideus geht der Luftwiderstand des Kehlkopfes hoch und

die Fläche der Öff nung nimmt ab. Während elektrischer Reizung des Krikoarytenoideus Posterior geht der Luftwiderstand des Kehlkopfes ab und die Fläche der Öffnung wird grösser. Wenn Krikothyroideus und Krikoarytenoideus Posterior nur selben gereizt werden, wird der Luftwiderstand am geringsten, und die Fläche der Öffnung die grösste. Die Synergie dieser Muskeln ist beschrieben und illustriert.

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SALIVARY-GLAND SCANNING WITH ^{99m}TECHNETIUM

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^{99m}Tc pertechnetate concentrates in the salivary glands and offers a possibility of scanning the glands. The difficulty of oral cavity contamination is overcome by giving 1 0 mg atropine intravenously before ^{99m}Tc administration.

Many salivary gland diseases are associated with functional disturbances. Their demonstration can often make a valuable contribution to the sometimes difficult diagnosis (Diamant, 1960) Such disturbances are, however difficult to establish clinically Deviations from the normal must be considerable if they are to be demonstrated by means of the quantitative and qualitative studies of the saliva hitherto used (Seige & Pfeiffer 1955 Seige, 1956 Enfors, 1962) The examinations are generally time-consuming, since the recordings must be made both at rest and under various degrees and types of salivary gland stimulation Moreover the saliva from the individual glands must be collected separately Consequently the need exists for a new functional test.

Since the radioactive isotope ^{99m}technetium (^{99m}Tc)—which was introduced in the early 1960s—is, in its pertechnetate form, concentrated in the salivary glands among other sites, it permits salivary gland scanning (Harper *et al.*, 1962) Technetium is an element in group VII and is formed when ^{99}molybdenum decomposes into ^{99m}technetium which, in turn decomposes into ^{99}technetium The half life of ^{99m}Tc is short, i e., only 6 hours The isotope emits a γ-radiation of 140 keV of which 8–9% is converted into β-radiation (Harper *et al* 1965 Smith, 1964)

In view of the short half life, the total body irradiation is low even if a relatively large quantity of ^{99m}Tc is injected. One millicurie gives a total body irradiation of about 20 millirad The thyroid gland and stomach, whose irradiation is greatest receive from 200–300 millirad. As a comparison, it can be mentioned that a thyroid scan with 50 microcurie of ^{131}I gives a total thyroid irradiation which is approximately 1000 times greater and that roentgenological examination of the stomach gives a dose over this organ that is about 10–20 times greater (Harper *et al.*, 1965)

Pertechnetate ^{99m}Tc is distributed in the body in almost exactly the same way as ^{131}I Thus, the isotope is concentrated in the gastric mucosa, and in the thyroid and salivary glands (Harper *et al.*, 1965 Andros *et al.*, 1965

harem *et al* 1967) Although occasional salivary gland scans have been published earlier the records have differed in appearance, due partly to the fact that ^{99m}Tc had collected in the oral cavity to a varying high degree, one of the results being that the concentration in the salivary glands varied appreciably (Börner *et al.*, 1965 Harden *et al* 1967) Harden stated that, on one occasion, premedication with 0.5 mg of atropine led to the amount of ^{99m}Tc decreasing in the oral cavity We have tried to devise a method in which salivary-gland scanning can be performed routinely with consistently good records, without contamination of the oral cavity by ^{99m}Tc.

MATERIAL AND METHODS

Pertechnetate ^{99m}Tc was obtained from ^{99}molybdenum piles delivered by AB Atomenergi, Studsvik, Sweden. The isotope was prepared daily and sterilized with the help of a Millipore® filter The procedure takes about 15 minutes. Totally 60 subjects were examined. Only a few of them had symptoms of salivary-gland disease.

In most cases, the examination was started with slow intravenous injection of 1 0 mg of atropine sulphate. After about 1 min, ^{99m}Tc was injected intravenously through the same cannula. The dose of ^{99m}Tc ranged from 1 to 6 millicurie generally 2.0 millicurie. The scanning, which was started 15 minutes after injection of ^{99m}Tc, occupied approximately 20 minutes. It was performed with a Nucab whole-body scanner provided with a 3-inch Na crystal and single-channel analyzer and an 11-hole collimator (rate 4 mm/second, distance between the lines 3 mm) In most cases, two or more scans were made in direct succession Since many of the subjects had difficulty in keeping their head still during the recording, the head was fixed in a stand constructed for the purpose

RESULTS

At the first examinations, made without preceding atropinization, good pictures of the salivary glands were sometimes obtained, but the scan had a varying appearance and was not easily reproducible. Records made in direct succession in the same subject showed that they rapidly changed in appearance and that considerable increasing activity occurred in the oral cavity (Fig 1) Since this increasing activity could be presumed to depend on transport of ^{99m}Tc with the saliva from the salivary glands to the oral cavity atropine was given to inhibit the salivary flow A small dose of atropine (0.5 mg intramuscularly) only occasionally prevented contamination of the oral cavity and the records continued to vary in appearance (Fig 2) When the dose was raised to 1.0 mg of atropine sulphate intravenously it was exceptional for the ^{99m}Tc to leak into the oral cavity despite the higher dose The salivary glands appeared consistently and the records

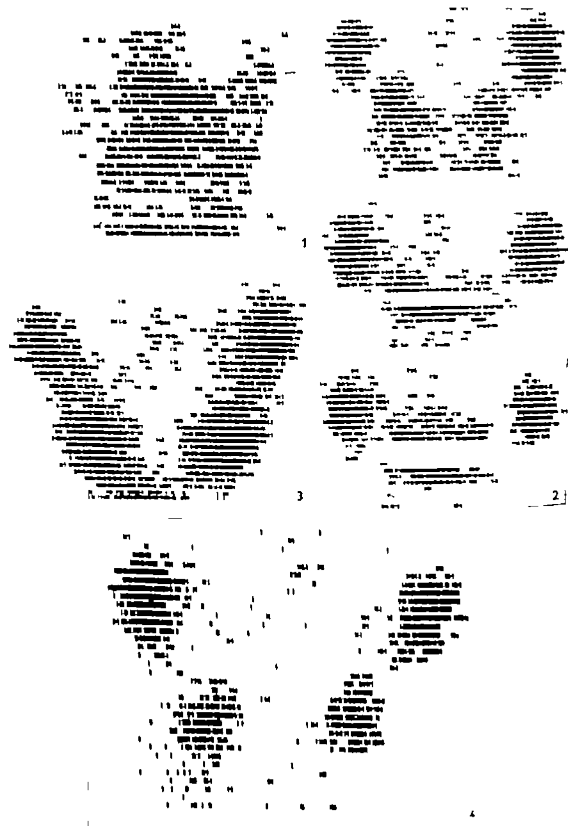


Fig 1 Without atropinizat n, contamination f the ral ca ity ft makes the record usel ss.

Fig 2. With unsatisf ctory trop izat n, cont minati n of th l ca ity occa l nally occurs. The record re taken l mmediat uccessi n (th first t th t p) I thi case mainly the submandibular glands ntribute t the ral contaminati

Figs. 3 and 4 With 1 0 mg f tropi lphat intra n usly co tam nati n of the ral cavity was regularly inhibited.

were of good quality (Figs. 3 and 4). The rare leakage into the oral cavity resulted in a decrease in the ^{99m}Tc content of the salivary glands (Fig. 2).

In the subjects without symptoms of salivary-gland disorders, the uptake of ^{99m}Tc was essentially the same bilaterally. Variations were, however, present between the uptake in the parotid and the submandibular glands (Figs. 3 and 4). The uptake also varied between different subjects. A possible explanation of a low uptake in the salivary glands, without contamination of the oral cavity, was that the atropine inhibited the glandular activity so greatly in certain individuals that the uptake of ^{99m}Tc was also affected. To rule out this possibility, a new recording was made in such cases without atropinization after a day or so had elapsed. In no case did we find that the low uptake could be ascribed to excessive atropine medication. Furthermore, a higher dose than 1.0 mg of atropine sulphate intravenously was never given.

DISCUSSION

The scanning method described can be used routinely to visualize the salivary glands. The standard dose—2.0 millicurie of ^{99m}Tc—which gives low total body irradiation, has proved sufficient to give good, uniform recordings. If greater resolution is desired to permit a more detailed analysis, the dose can be raised to 8 millicurie and the rate of scanning be decreased. The degree of density of the records is dependent partly on the total quantity of ^{99m}Tc injected, and partly on the relative concentration of the isotope in the parenchyma of the salivary glands. The uptake in these glands can be presumed to vary with their different functional and pathological state. In occasional examination of patients with symptoms of salivary-gland disease, records differing from those in healthy subjects were obtained. It is possible that this scanning method may assume importance for the evaluation of such pathological conditions.

ZUSAMMENFASSUNG

^{99m}Tc-pertechnetate wird in den Speicheldrüsen akkumuliert und bietet ein Möglichkeit, diese Drüsen szintigraphisch zu untersuchen. Die Schwierigkeit der Mundhöhlen-Kontaminierung überwindet man, indem man 1,0 mg Atropin intravenös vor der Verabreichung von ^{99m}Tc gibt.

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Jämförande antibakteriella aktivitetsbestämningar har bekräftat de kliniska iakttagelserna att V penicillin fortfarande försvarar sin plats som förstahandspreparat vid penicillinbehandling. För att säkerställa terapisvar även vid infektioner förorsakade av stammar med något mindre känslighet för penicillin har man mer och mer övergått till högre dosering.

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DEKLARATION

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Phenoximethylpenicillinkalium 0,4 g
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INDIKATIONER

Alla infektionssjukdomar förorsakade av penicillinkänsliga bakterier

KONTRAINDIKATION

Penicillinöverkänslighet

DOSERING

Vuxna: 4 tabletter per dag (1 på morgonen, 1 vid middagstiden och 2 till natten)

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Glas à 20 30 40 100 och 250 tabletter
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Tabletter for vuxna — Mixtur for barn

DEKLARATION
10 ml m t resp. 1 t bl tt in håll
Cinnarizin 10 mg + f ylprop lamin .5 mg

DOSERING
V xna 1 tabl 3 ggr d gl
B rn 5—1 år 1 tabl ll 10 ml mixtur morg n och k äll
B rn 1— å 5 ml mixtur m rg n h k äll

FÖRPACKNINGAR
Mixt 100 och .50 ml
Tablett r 25, 100 ch 2×0 t

Se f ö rigt FASS® 1969

